FINAL REPORT

on

TASK 89-09

to

Department of the Army
U.S. Army Medical Research
and Materiel Command
Fort Detrick, Frederick, MD 21702-5012

November, 1994

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FINAL REPORT

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on

TASK 89-09
A COMPARISON BETWEEN THE RHESUS MONKEY AND THE HUMAN ON THE EFFECT OF ATROPINE ON THE ELECTROENCEPHALOGRAM

VOLUME I

to

Department of the Army
U.S. Army Medical Research
and Materiel Command
Fort Detrick, Frederick, MD 21702-5012

November, 1994

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Statistical models were developed to analyze the effects of atropine on brain activity. Brain activity was measured from the spectral EEG waveforms as a function of the percent power in the first five frequency bands. The statistical analyses indicated that:

1. High and medium doses of atropine were associated with a statistically significant increase in percent power in the first freqency band, with an decrease in percent power in the fifth frequency band, which persisted through the 600 minute time point.

2. The low atropine dose was associated with a statistically significant increase in percent power in the first freqency band, and a decrease in percent power in the fifth frequency band, at the 30 and 90 minute time points. Following the low atropine dose, there were no significant measurable effects of atropine on EEG waveforms at the 300 and 600 minute time points.

3. There were no significant measurable trends in the EEG waveforms for monkeys injected with the saline control.

4. The effect of atropine on brain activity (as measured by EEG) appears to diminish with respect to time since dosing.

A draft report of the experimental results was reviewed by Dr. John R. Hughes, Director of the Department of Neurophysiology at the University of Illinois, Chicago Medical Center. Dr. Hughes concluded that a 15-fold increase in sensitivity in the measurement of atropine effects was attributable to the use of computerized EEG analyses. Dr. Hughes believes that high percent power in the first frequency band, and corresponding decrease in percent power in the fifth frequency band, are associated with a decrease in brain activity, and vice versa. Based on extrapolation of these results from monkeys, Dr. Hughes concluded that a reduction in brain activity in man could appear at an atropine dose of 0.003 mg/kg. This extrapolation is comparable to previously reported data which demonstrated divided attention in man at an atropine dose of 0.007 mg/kg. The slowing in brain activity predicted to occur at this dose range of atropine (0.003 to 0.007 mg/kg) is not expected to seriously impair the soldier. Based on his review of the literature, Dr. Hughes believes that an atropine dose of 0.012 mg/kg in man would produce a substantially larger reduction in brain activity, associated with an increase in coordination errors, and could significantly impair the ability of a soldier to perform complex tasks. Furthermore, Dr. Hughes concluded from his review of the literature that an atropine dose of 0.028 mg/kg could affect helicopter flight performance in some individuals, and an atropine dose of 0.040 mg/kg would likely place a soldier on the battlefield in jeopardy.

EXECUTIVE SUMMARY

In 1987 as part of MREF Task 87-32, a study was conducted to examine the effects of atropine on the brain activity of 15 rhesus monkeys. Each animal was administered three doses of atropine (High = 0.4 mg/kg, Medium = 0.2 mg/kg, and Low = 0.1 mg/kg) and the electroencephalogram (EEG) of each animal was monitored at each dose of atropine. The experimentation employed a crossover design for the order of dosing of each animal, and used a washout period of approximately one month between atropine doses. Approximately four months after the conclusion of the atropine dosing experiments, brain wave activities of each animal were monitored following the administration of a saline solution. In August 1992, a preliminary investigation of data from three monkeys demonstrated a statistically significant difference in EEG activity between the high atropine dose and saline control experiments. Following the completion of the preliminary study, a more thorough analysis was conducted to investigate the dose-response relationship between atropine and EEG waveforms.

The experimental results from six of the fifteen rhesus monkeys were selected for a more thorough examination of the spectral EEG waveforms. Two of the monkeys selected were from the original three studied, and the other four monkeys were selected to complement the data from these two animals, resulting in a balanced fraction of the crossover experimental design. Samples of EEG for spectral analysis were obtained at the following time points for each animal: Baseline and 30, 90, 300, and 600 minutes following dosing. The baseline EEG sample was obtained prior to dosing in each experiment, and serves as a basis for comparison.

Statistical models were developed to analyze the effects of atropine on brain activity. Brain activity was measured from the spectral EEG waveforms as a function of the percent power in the first five frequency bands. The statistical analyses indicated that:

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TASK 89-09: A COMPARISON BETWEEN THE RHESUS MONKEY AND THE HUMAN ON THE EFFECT OF ATROPINE ON THE ELECTROENCEPHALOGRAM

1.0 INTRODUCTION

The experimental portion of Task 87-32, "Evaluation of the Pharmacokinetic Profile of Atropine and its Effect on Various Physiologic Parameters in the Rhesus Monkey," was performed at Battelle for the U.S. Army Medical Research and Materiel Command (USAMRMC) between October 1987 and April 1988 (Joiner, R.L., Hassler, C.R., Dill, G.S., Final Report on Task 87-32, March 1990). The study was designed to investigate the serum kinetics of high doses of atropine and the effects of atropine on heart rate, electrocardiogram (ECG), rectal temperature, pupil diameter, and electroencephalogram (EEG). The rhesus monkey was selected for this experiment because there is considerable documentation that the monkey is predictive of effects in man, and historically the rhesus monkey has been accepted as a non-human primate model for man. Monkeys and atropine in a citrate buffer (approximately 2.4 mg atropine free base/mL) were provided by the U.S. Army. Following health examinations and quarantine, monkeys were acclimated to restraint and to placement in an isolation chamber. Each animal's scalp was tattooed to mark placement of subcutaneous platinum EEG electrodes. On each day of experimentation, a catheter was placed in a saphenous or femoral vein of one monkey to collect timed blood samples for atropine concentration determinations, and ECG and EEG electrode placement areas were clipped of hair. The monkey was then placed in a restraint chair, ECG and EEG electrodes applied, and a thermometer positioned in the rectum. The prepared monkey in the restraint chair was moved into the isolation chamber, and when the animal appeared calm, baseline ECG and EEG recordings, and pupil diameter and rectal temperatures measurements were taken and then a blood sample was collected via the catheter. The monkey was injected in the distal portion of the caudal muscles of the thigh with the atropine dose preselected for that animal on that day. Blood samples and the selected physiologic parameters were measured periodically for up to 10 hours. At all designated times, the collection of blood samples took precedence over collection of physiologic data.

Analog records of resting and light-evoked response data were recorded on a Nicolet 1A98 21-channel EEG recorder. Digitized records of resting EEG data were collected with a Nicolet Pathfinder 1, and digitized data were transferred to a streaming tape. Analog paper and digitized records of approximately 3 minutes of resting EEG were taken at collection times more than 15 minutes following atropine injection.

Each of 15 monkeys was administered three doses of atropine (High = 0.4 mg atropine free base/kg, Medium = 0.2 mg atropine/kg, and Low = 0.1 mg atropine/kg). A crossover design was implemented for these atropine experiments with a washout period of approximately one month between dosings. Approximately four months after atropine dosing, each animal was injected with 0.5 mL physiological saline only, and monitored for EEG changes as in the atropine experiments.

An August 1992 pilot investigation of the EEG data was conducted under MREF Task 89-09. This task was designed to analyze EEG waveforms of monkeys after they were treated with atropine and to correlate these findings with effects in humans. The brain wave data of each of three monkeys (122X, 257D, D275) following the high atropine dose and following the saline control dose were filtered and smoothed using spectral analysis. The smoothed data were then statistically analyzed to assess the effects of atropine on percent power in different frequency bands. Individual analysis of variance models were fitted to the data for animals D275 and 122X to determine the effect of atropine on the average shift in percent power from baseline. Because data from the control experiment were not available for animal 257D, it was not possible to compare the results between the high atropine dose and control experiments for this animal. The overall conclusion of the statistical analysis for the pilot study was that there were significant shifts in percent power from baseline for the high dose of atropine when compared to the control experiments.

Following the pilot study, additional animals and dosing experiments were selected for a more thorough examination of the spectral EEG waveforms. This analysis of the EEG waveforms included all three atropine doses and the saline control for six animals. Four additional animals were selected to complement the experimental design of the dosing patterns of animals 122X and D275, producing a balanced fraction of the original crossover analysis. This report summarizes the statistical analysis of the spectral EEG waveforms for animals 038D, 122X, 4E5, D275, D345, and E109.

2.0 EEG DATA REDUCTION

2.1 SAMPLING EEG DATA

Samples of EEG for spectral analysis were obtained at the following time points in the recording sessions for each animal: 0, 30, 90, 300, and 600 minutes following dosing. These time points coincided with the drawing of blood samples. The periods of time considered for EEG sampling varied depending on the time point as follows:

- 0-Minute Time Point. For the baseline sample of EEG, any period of time prior to administration of atropine was used as long as it did not coincide with a period of sensory stimulation or a period during which a blood sample was being drawn.

- 30- and 90-Minute Time Points. The EEG sampling period started with the closing of the chamber door following blood collection at that sampling time and ended at the onset of sensor stimulation at the next data collection epoch.

- 300- and 600-Minute Time Points. The EEG sampling period consisted of 20 minutes before the opening of the chamber door for drawing the blood sample and 20 minutes after the chamber door was closed again.

2.2 BEHAVIORAL CONDITIONS FOR OBSERVING EEG DATA

Within each EEG sampling period, the video record of the animal was reviewed to identify time periods during which the animal was awake and relaxed so EEG data of acceptable quality could be obtained. The video review gave first priority to the time period immediately following the blood sampling. However, if the initial review did not yield sufficient time periods with acceptable EEG data, the review was expanded to include additional times within the sampling periods described above.

The following criteria were used to define acceptable sampling periods for each animal:

- Animal quiet without signs of overt agitation or excessive movement, such as shaking or jerking of the head, vocalizations, etc. Slow, purposeful head movements and/or eye movements were tolerated.

- Eyes open. The ideal situation was to have the eyes fully and continuously open with the obvious appearance of alertness. Lesser states of alertness, however, were tolerated. The following conditions were considered acceptable:

  -- Eyes half-closed;
  -- Eyes fully closed for 5 seconds or less;
  -- A combination of eyes half-closed and eyes fully closed lasting 10 seconds or less.

For each sampling period, a paper tracing of the EEG was reproduced on a polygraph from the tape-recorded data. This EEG tracing included the time periods containing acceptable behavior identified in the video review.

2.3 ELIMINATION OF ARTIFACTS FROM SAMPLED EEG

For each sampling period, the EEG tracing marked with periods of acceptable behavior was reviewed to identify two-second segments (or "windows") of continuous EEG signal. Ideally, these segments were completely free of artifact, but some contamination was tolerated. The major criterion for accepting a given segment was a relative lack of baseline deviation within the EEG tracing. This criterion was applied at three levels of strictness. In decreasing order of strictness, these levels were

a. No baseline deviation at all.
b. Baseline deviation with a rise time greater than 1 second.
c. Baseline deviation with a rise time greater than 0.5 second.

Thirty two-second segments which met at least one of the above levels of the baseline criteria were identified within each sampling period. Thirty two-second segments at each time period were thought to be the minimum necessary to obtain an accurate estimate of an animal's EEG. If 30 segments could not be obtained, then no segments for that sampling period were identified.

2.4 SPECTRAL ANALYSIS

For each two-second window, digital filtering (at 1.0 Hz high pass) and spectral analysis was done on the EEG waveforms using a Nicolet Pathfinder I Electrodiagnostic System. Four bipolar EEG channels were analyzed:

- Channel 1 (Left Frontal/Central Motor - Right Frontal/Central Motor)
- Channel 2 (Left Parietal - Right Parietal)
- Channel 3 (Left Frontal/Central Motor - Left Occipital)
- Channel 4 (Right Frontal/Central Motor - Right Occipital).

Spectral analysis integration was performed across each of the following frequency bands:

Band	Hz
1	1-4
2	4-8
3	8-13
4	13-18
5	18-25
6	25-30
7	30-70
8	59-61
9	1-25

The reduced EEG data were electronically transferred from the Nicolet pathfinder to the Battelle mainframe computer system for statistical analysis.

3.0 DATA SUMMARY AND ANALYSIS METHODS

Tasks performed in the summary and analysis of reduced EEG data were as follows:

- Compute descriptive statistics (mean, standard deviation, standard error, sample size) of four parameters (total power, percent power, mean frequency, peak frequency) within each combination of animal, atropine dose, time period, frequency band, window portion (windows 1-10, 11-20, 21-30), and channel.

- Plot the mean values for percent power over all 30 sampling windows versus frequency band for each time period, noting trends in the means across the frequency bands and how the trends differ among the time periods. Prepare and compare plots for each animal, atropine dose, and channel.

- Determine whether significant differences from baseline (time 0) occur across animals in percent power for the lowest frequency band for each time period. Also determine whether significant differences exist in the mean baseline shift between atropine dosing and saline dosing.

- Determine for each animal whether significant differences from baseline (time 0) occur in percent power for the lowest frequency band for each time period. Also determine whether significant differences exist in the mean baseline shift between atropine dosing and saline dosing.

Each animal received the three doses of atropine according to a simple crossover design prior to receiving the saline dose. The three atropine doses were given at approximately one-month intervals, while the saline dose was given approximately four months following the last atropine dose. Data from four different EEG leads (channels) and up to eight frequency bands were monitored. Within each frequency band and channel, study directors selected 30 sampling windows within each time period from which data for the following four parameters were collected:

- Mean frequency
- Peak frequency
- Total power
- Percent power

It was not possible to obtain sufficient data from all six animals for each time period and dose for complete data summary and analysis. Table 1 displays the available data and the order of dosing for each of the six animals included in this table. Baseline comparisons were possible within a dose level only when data were reported at the 0 minute time period. Comparisons with the saline group are possible only when saline dose data were reported.

Data representing the four parameters of interest were extracted and placed into SAS® datasets for data summary and analysis. Mean and peak frequency were observed for five frequency bands, as well as a global band covering the entire range of frequencies (0.5 - 19.5 Hz) represented by these five bands. Only positive frequency data were accepted. Total power was collected for eight frequency bands covering all frequencies. Percent power, collected within the same five frequency bands for which mean and peak frequency were collected, represents that band's percentage of the power in those five frequency bands. Power in the highest frequency bands (bands 6 through 8) was not analyzed due to high interference levels.

3.1 DESCRIPTIVE STATISTICS

Means, standard deviations, and standard errors of the mean were calculated for each of the four data parameters in Section 3.0 according to the following combinations of factors:

- Animal
- Channel
- Time period
- Frequency band
- Window portion within a time period (first set of ten windows, second set of ten windows, third set of ten windows) .

The 30 two-minute EEG segments selected for analyses were divided into three portions so that differences between segments selected could be statistically evaluated. Means and standard deviations calculated for each window portion were based on ten data

TABLE 1. AVAILABLE DATA FOR THE 1993 MREF EEG DATA ANALYSIS (TASK 89-09)

Animal ID	Dose (mg/kg)	Order of Dosing	Time Following Dosing				
			0 min.	30 min.	90 min.	300 min.	600 min.
038D	0.0	4					
	0.1	1	—	—	—		
	0.2	3	—	—	—		
	0.4	2		—			
122X	0.0	4		—			
	0.1	3		—			
	0.2	2		—			
	0.4	1			—		
4E5	0.0	4					
	0.1	2					
	0.2	1		—	—	—	
	0.4	3	—	—	—	—	—
D275	0.0	4					
	0.1	2					
	0.2	3					
	0.4	1			—		
D345	0.0	4					
	0.1	1	—	—	—		
	0.2	2	—	—			
	0.4	3		—	—	—	—
E109	0.0	4	—				
	0.1	3					
	0.2	1					
	0.4	2		—	—	—	—

Each unshaded cell represents 30 data points. Shaded cells denote no data are available.

points (one data point per two-second window). In addition, for each combination of animal, channel, time period, and frequency band, the grand mean for each window was computed by averaging the three mean values calculated for each window portion. Tables of the mean parameter values, along with standard deviations, standard errors of the mean, and sample sizes, are found in Appendix A. For a given animal monitored within a specific dose experiment (atropine or saline), one table of descriptive statistics exists for each data parameter and channel.

3.2 MODELING THE EFFECTS OF ATROPINE ON BRAIN ACTIVITY

Of primary interest in statistical analysis of the EEG data were total power and percent power. It was hypothesized that the effects of atropine were to shift power levels from higher to lower frequency bands, thus increasing the total power and percent power in the lower bands. A plot of power versus frequency band should provide a visual assessment of the effect of atropine on the distribution of power across the frequency bands. The grand means of percent power, presented in the last column group in the descriptive statistics tables of Appendix A, are plotted versus frequency band for each time period simultaneously. Separate plots, located in Appendix B, were prepared for each combination of animal, channel, and atropine dose. Plotting symbols representing means for the same period of time are connected by lines in these plots so that trends can be more easily identified. Thus the effects of atropine on the distribution of total and percent power can be inferred by comparing the plots for saline and atropine dosing for a given animal, and by observing how trends change across the various periods of time within a plot.

3.2.1 Inter-Animal Analysis

While the plots provide a visual assessment of trends in percent power across the first five frequency bands for each period of time and dosing experiment, the effect of atropine on power was assessed quantitatively by statistical modelling techniques. The

statistical analysis focused on evaluating average shifts in percent power across all dosing experiments. As power shifts from high to low frequencies, the percent of total power in the lower bands among the first five frequency bands will increase. Thus an analysis of percent power across all five frequency bands demonstrates the extent of shifts in power among the frequency bands and how these shifts differ among the dosing experiments and time.

A statistical approach was developed to investigate the relationship between atropine dose and percent power among the first five frequency bands averaged across all six animals. This approach involved the use of a mixed model which included a variance component for the animal effect. This random animal effect allows the statistical model to compensate for the fact that the animals may behave somewhat differently in each dose experiment. The fixed effects in this model allow for an assessment of the effects of dose over time as measured by percent power. The model appears as follows:

$$Y_{aijk} = \mu + D_i + B_j + (DB)_{ij} + RI_{ai} + e_{aijk}, \quad \begin{aligned} a &= 1,\ldots,6; \\ i &= 1,\ldots,4; \\ j &= 1,\ldots,5; \\ k &= 1,\ldots,30 \end{aligned} \tag{1}$$

where, Y_{aijk} is the k^{th} percent power reading in the first frequency band for the i^{th} dosing experiment and the j^{th} time period within the dosing experiment for the a^{th} animal,

μ is an overall constant,

D_i is the effect due to the i^{th} dosing experiment ($i=1$ is saline dosing, $i=2$ is low dose, $i=3$ is medium dose, and $i=4$ is high dose),

B_j is the effect due to the j^{th} time period ($j=1$ is baseline, $j=2$ is 30 minutes post-dosing, $j=3$ is 90 minutes post-dosing, $j=4$ is 300 minutes post-dosing, and $j=5$ is 600 minutes post-dosing),

$(DB)_{ij}$ is the interaction effect between the i^{th} dosing experiment and the j^{th} time period,

RI_{ai} is the random effect for the i^{th} dosing experiment on animal a, and

e_{aijk} is the error not attributable to the model.

This model was fit separately for each channel to the percent power data in each of the first five frequency bands through restricted maximum likelihood estimation. F-tests were implemented in the analysis of variance to determine whether effects of atropine dosing and time were statistically significant, and whether the extent of the atropine effect significantly differed between time periods. Statistical tests (Likelihood Ratio and Wald tests) were performed to assess the statistical significance of the variance component associated with the random animal effect. In addition, linear contrasts of model parameters were performed to determine:

- Those post-dosing periods of time with significant shifts in percent power from baseline within a given dosing experiment,
- The significance of the difference in shift from baseline between the atropine and saline dosing experiments.

Table 2 summarizes the average shifts from baseline in percent power in the first frequency band. The average shift from baseline (labelled "Est.") is presented for each combination of channel, dose experiment, and time period. A positive average shift indicates that percent power averaged higher in the post-dosing time compared to baseline. Standard errors in the estimated average shift are presented alongside the mean shifts (labelled "S.E."). The statistical significance of each average shift is assessed through the p-value (labelled "p") calculated by the statistical model. P-values at 0.05 or lower indicate that the shift from baseline is significantly different from zero at the 5 percent level, i.e., the probability of this occurring by chance is 5 percent or less.

In addition to analyzing the shift in percent power in the first frequency band between baseline and subsequent post-dosing time points, the magnitudes of the average shift from baseline were compared between the saline control and atropine experiments. Estimated differences in baseline shift from the saline control, associated standard errors, and corresponding p-values are displayed in Table 3.

TABLE 2. ESTIMATED DIFFERENCE FROM BASELINE IN PERCENT POWER IN THE FIRST FREQUENCY BAND, WITH STANDARD ERROR AND LEVEL OF SIGNIFICANCE FROM ZERO, ACROSS ALL STUDY ANIMALS SIMULTANEOUSLY

	Dose Level	30 min. post-dose			90 min. post-dose			300 min. post-dose			600 min. post-dose		
		Est.	S.E.	p	Est.	S.E.	p	Est.	S.E.	p	Est.	S.E.	p
Channel 1	Saline	-0.40	1.63	0.807	-4.96	1.54	0.001	-6.92	1.54	0.000	-2.28	1.54	0.138
	Low	14.76	1.95	0.000	11.03	1.78	0.000	3.76	1.69	0.026	4.60	1.69	0.007
	Medium	16.77	2.28	0.000	18.26	1.87	0.000	16.54	1.81	0.000	13.51	1.68	0.000
	High	25.92	2.33	0.000	17.82	3.09	0.000	19.17	2.01	0.000	17.52	2.01	0.000
Channel 2	Saline	2.05	1.36	0.132	-1.75	1.28	0.172	-2.59	1.28	0.044	1.22	1.28	0.343
	Low	8.93	1.63	0.000	7.18	1.48	0.000	1.93	1.42	0.174	2.43	1.42	0.086
	Medium	13.74	1.91	0.000	11.95	1.57	0.000	9.49	1.52	0.000	8.32	1.41	0.000
	High	23.43	1.96	0.000	19.68	2.60	0.000	20.13	1.70	0.000	14.27	1.70	0.000
Channel 3	Saline	1.67	1.31	0.203	-2.47	1.24	0.046	-3.03	1.24	0.014	0.94	1.24	0.447
	Low	10.28	1.57	0.000	6.43	1.43	0.000	-1.71	1.37	0.211	-2.33	1.37	0.088
	Medium	11.24	1.84	0.000	15.97	1.51	0.000	11.15	1.46	0.000	8.10	1.36	0.000
	High	20.29	1.89	0.000	29.11	2.51	0.000	19.64	1.64	0.000	14.25	1.64	0.000
Channel 4	Saline	3.18	1.35	0.019	0.85	1.28	0.505	-1.57	1.28	0.220	3.26	1.28	0.011
	Low	10.82	1.62	0.000	7.96	1.48	0.000	0.00	1.41	0.998	1.51	1.41	0.284
	Medium	11.41	1.90	0.000	19.99	1.56	0.000	15.57	1.51	0.000	11.17	1.40	0.000
	High	19.89	1.95	0.000	23.46	2.58	0.000	20.27	1.69	0.000	15.18	1.69	0.000

1. "Est." is the estimated difference from the baseline (pre-dose) time period in percent power in the first frequency band for the given dose level/time period combination. "S.E." is the estimated standard error in this estimate. "p" is the significance level for testing whether this difference from baseline is significantly different from zero.

2. "Saline" is 0.0 mg/kg atropine, "Low" is 0.1 mg/kg atropine, "Medium" is 0.2 mg/kg atropine, and "High" is 0.4 mg/kg atropine.

TABLE 3. ESTIMATED DIFFERENCE IN BASELINE SHIFT BETWEEN EACH DOSE LEVEL AND SALINE CONTROL DOSE FOR PERCENT POWER IN THE FIRST FREQUENCY BAND, WITH STANDARD ERROR AND LEVEL OF SIGNIFICANCE FROM ZERO, ACROSS ALL ANIMALS SIMULTANEOUSLY

	Dose Level	30 min. post-dose			90 min. post-dose			300 min. post-dose			600 min. post-dose		
		Est.	S.E.	p	Est.	S.E.	p	Est.	S.E.	p	Est.	S.E.	p
Channel 1	Low	15.16	2.54	0.000	15.99	2.35	0.000	10.68	2.29	0.000	6.88	2.29	0.003
	Medium	17.17	2.80	0.000	23.22	2.42	0.000	23.45	2.38	0.000	15.79	2.28	0.000
	High	26.32	2.84	0.000	22.77	3.46	0.000	26.09	2.53	0.000	19.81	2.53	0.000
Channel 2	Low	6.88	2.12	0.001	8.94	1.96	0.000	4.52	1.91	0.018	1.21	1.91	0.526
	Medium	11.69	2.35	0.000	13.71	2.03	0.000	12.08	1.99	0.000	7.10	1.91	0.000
	High	21.38	2.39	0.000	21.44	2.90	0.000	22.72	2.13	0.000	13.05	2.13	0.000
Channel 3	Low	8.61	2.04	0.000	8.90	1.89	0.000	1.32	1.84	0.474	-3.27	1.84	0.076
	Medium	9.57	2.26	0.000	18.44	1.95	0.000	14.18	1.92	0.000	7.16	1.84	0.000
	High	18.62	2.30	0.000	31.58	2.80	0.000	22.67	2.05	0.000	13.31	2.05	0.000
Channel 4	Low	7.64	2.11	0.000	7.11	1.95	0.000	1.57	1.90	0.409	-1.75	1.90	0.358
	Medium	8.23	2.33	0.000	19.14	2.01	0.000	17.14	1.98	0.000	7.91	1.90	0.000
	High	16.71	2.37	0.000	22.61	2.88	0.000	21.84	2.12	0.000	11.92	2.12	0.000

1. For percent power in the first frequency band, "Est." is the estimated difference in the baseline shifts (Table 8) between the given dose level and the saline control dose. "S.E." is the estimated standard error in this estimate. "p" is the significance level for testing whether this difference from the saline control dose is significantly different from zero.

2. "Saline" is 0.0 mg/kg Atropine, "Low" is 0.1 mg/kg Atropine, "Medium" is 0.2 mg/kg Atropine, and "High" is 0.4 mg/kg Atropine.

Tables 4 to 7 display the baseline shifts in percent power within each of the first five frequency bands for each channel. Tables 8 to 11 present the difference in baseline shifts between atropine and saline control experiments across all five frequency bands for all four channels. These tables illustrate how percent power shifts from one frequency band to another across the animals. While these tables do not present the standard error associated with each parameter estimate, they do indicate whether the estimate is significantly different from zero at the 0.05 and 0.01 levels.

3.2.2 Intra-Animal Analysis

Statistical models were also fit separately to the data from each animal to explore the effect of atropine dose and time on each animal. The following statistical model was used to identify how percent power in the first frequency band differs among dosing experiments and time periods:

$$Y_{ijk} = \mu + D_i + B_j + (DB)_{ij} + e_{ijk},\ i = 1,\ldots,4;\ j = 1,\ldots,5;\ k = 1,\ldots,30 \qquad (2)$$

where, Y_{ijk} is the k^{th} percent power reading in the first frequency band for the i^{th} dosing experiment and the j^{th} time period within the dosing experiment,

μ is an overall constant,

D_i is the effect due to the i^{th} dosing experiment ($i=1$ is saline dosing, $i=2$ is 0.1 mg/kg dose, $i=3$ is 0.2 mg/kg dose, and $i=4$ is 0.4 mg/kg dose),

B_j is the effect due to the j^{th} time period ($j=1$ is baseline, $j=2$ is 30 minutes post-dosing, $j=3$ is 90 minutes post-dosing, $j=4$ is 300 minutes post-dosing, and $j=5$ is 600 minutes post-dosing),

$(DB)_{ij}$ is the interaction effect between the i^{th} dosing experiment and the j^{th} time period, and

e_{ijk} is the error not attributable to the model.

TABLE 4. ESTIMATED DIFFERENCE FROM BASELINE IN PERCENT POWER ACROSS ALL 5 FREQUENCY BANDS IN CHANNEL 1, BASED ON MODEL (1)

	Dose Level	30 min. post-dose	90 min. post-dose	300 min. post-dose	600 min. post-dose
		Est.	Est.	Est.	Est.
Band 1	Saline	-0.40	-4.96**	-6.92**	-2.28
	Low	14.76**	11.03**	3.76*	4.59**
	Medium	16.77**	18.26**	16.54**	13.51**
	High	25.92**	17.82**	19.17**	17.52**
Band 2	Saline	0.42	-1.38	-1.06	1.25
	Low	0.31	1.85*	-3.06**	-0.04
	Medium	0.92	2.93**	1.17	0.11
	High	5.93**	7.46**	6.72**	4.55**
Band 3	Saline	-0.77	-0.82	-0.69	-0.70
	Low	-3.94**	-4.60**	-5.66**	-4.04**
	Medium	-6.24**	-4.27**	-5.94**	-6.10**
	High	-7.44**	-8.66**	-7.11**	-6.85**
Band 4	Saline	0.23	0.85	1.22	0.61
	Low	-3.41**	-3.57**	1.07	-0.40
	Medium	-5.60**	-7.74**	-6.75**	-5.89**
	High	-8.97**	-2.49	-7.27**	-6.30**
Band 5	Saline	0.30	6.15**	7.31**	1.43
	Low	-8.36**	-5.20**	3.81**	-0.37
	Medium	-5.46**	-9.71**	-5.60**	-2.39
	High	-14.82**	-12.07**	-11.02**	-7.56**

* Denotes statistical significance at the p = 0.05 level.
** Denotes statistical significance at the p = 0.01 level.

TABLE 5. ESTIMATED DIFFERENCE FROM BASELINE IN PERCENT POWER ACROSS ALL 5 FREQUENCY BANDS IN CHANNEL 2, BASED ON MODEL (1)

	Dose Level	30 min. post-dose	90 min. post-dose	300 min. post-dose	600 min. post-dose
		Est.	Est.	Est.	Est.
Band 1	Saline	2.05	-1.75	-2.59*	1.22
	Low	8.93**	7.18**	1.92	2.43
	Medium	13.74**	11.95**	9.49**	8.32**
	High	23.43**	19.68**	20.13**	14.27**
Band 2	Saline	0.05	-1.27	-0.77	3.90**
	Low	2.14*	1.76	-0.77	-1.30
	Medium	0.90	4.63**	0.87	-0.27
	High	5.64**	7.23**	6.47**	7.59**
Band 3	Saline	-1.30	-1.21	-1.59*	-2.89**
	Low	-5.53**	-6.17**	-6.89**	-5.49**
	Medium	-7.03**	-4.72**	-5.93**	-6.76**
	High	-6.85**	-6.72**	-6.37**	-3.90**
Band 4	Saline	-0.11	1.36	1.88*	-1.23
	Low	-1.38	-2.27*	1.32	1.15
	Medium	-4.10**	-5.57**	-5.13**	-3.60**
	High	-8.85**	-6.24**	-8.85**	-8.69**
Band 5	Saline	-0.84	2.78**	2.97**	-0.57
	Low	-4.60**	-0.80	4.32**	3.04**
	Medium	-3.38**	-6.72**	0.25	1.63
	High	-13.27**	-12.16**	-11.20**	-8.55**

* Denotes statistical significance at the $p = 0.05$ level.
** Denotes statistical significance at the $p = 0.01$ level.

TABLE 6. ESTIMATED DIFFERENCE FROM BASELINE IN PERCENT POWER ACROSS ALL 5 FREQUENCY BANDS IN CHANNEL 3, BASED ON MODEL (1)

	Dose Level	30 min. post-dose Est.	90 min. post-dose Est.	300 min. post-dose Est.	600 min. post-dose Est.
Band 1	Saline	1.67	-2.47*	-3.03*	0.94
	Low	10.28**	6.43**	-1.71	-2.33
	Medium	11.24**	15.97**	11.15**	8.10**
	High	20.3**	29.11**	19.64**	14.24**
Band 2	Saline	0.22	-1.08	0.13	0.89
	Low	-0.52	0.15	-3.57**	-2.13*
	Medium	1.46	4.60**	1.79	-0.13
	High	3.44**	2.61	2.12	1.41
Band 3	Saline	-2.10**	-1.53*	0.22	-1.15
	Low	-2.99**	-2.57**	-4.47**	-3.07**
	Medium	-4.48**	-5.29**	-6.61**	-6.77**
	High	-3.66**	-6.65**	-6.09**	-4.01**
Band 4	Saline	-0.90	0.11	0.31	0.08
	Low	-1.60	-0.19	3.88**	2.90**
	Medium	-3.87**	-6.90**	-3.88**	-3.15**
	High	-7.88**	-6.32**	-5.27**	-4.46**
Band 5	Saline	0.93	4.84**	2.26*	-0.44
	Low	-5.56**	-4.09**	5.93**	4.43**
	Medium	-4.13**	-8.83**	-2.92**	1.27
	High	-11.81**	-17.12**	-9.95**	-6.16**

* Denotes statistical significance at the p = 0.05 level.
** Denotes statistical significance at the p = 0.01 level.

TABLE 7. ESTIMATED DIFFERENCE FROM BASELINE IN PERCENT POWER ACROSS ALL 5 FREQUENCY BANDS IN CHANNEL 4, BASED ON MODEL (2)

	Dose Level	30 min. post-dose	90 min. post-dose	300 min. post-dose	600 min. post-dose
		Est.	Est.	Est.	Est.
Band 1	Saline	3.18*	0.85	-1.57	3.26*
	Low	10.82**	7.96**	0.00	1.51
	Medium	11.41**	19.99**	15.57**	11.17**
	High	19.89**	23.46**	20.27**	15.18**
Band 2	Saline	1.09	0.33	0.06	2.36**
	Low	0.61	0.08	-2.44*	-1.85
	Medium	1.12	3.79**	1.49	0.90
	High	5.46**	1.82	4.99**	2.87**
Band 3	Saline	-0.13	-0.51	0.46	0.36
	Low	-2.90**	-2.35**	-4.03**	-2.91**
	Medium	-4.60**	-4.57**	-7.44**	-7.08**
	High	-5.44**	-7.48**	-7.14**	-5.30**
Band 4	Saline	-0.26	-0.08	0.53	0.53
	Low	-2.45**	-1.35	2.97**	1.16
	Medium	-4.60**	-8.75**	-5.79**	-5.06**
	High	-7.46**	-4.55**	-7.18**	-5.04**
Band 5	Saline	-4.09**	-0.73	0.39	-6.14**
	Low	-6.61**	-4.74**	3.36**	1.66
	Medium	-3.21*	-11.01**	-4.42**	-0.75
	High	-11.86**	-11.43**	-10.14**	-6.56**

* Denotes statistical significance at the p = 0.05 level.
** Denotes statistical significance at the p = 0.01 level.

TABLE 8. ESTIMATED DIFFERENCE IN BASELINE SHIFT BETWEEN EACH LEVEL OF ATROPINE DOSE AND SALINE CONTROL DOSE FOR PERCENT POWER IN ALL 5 FREQUENCY BANDS BASED ON MODEL (1) IN CHANNEL 1

	Dose Level	30 min. post-dose	90 min. post-dose	300 min. post-dose	600 min. post-dose
		Est.	Est.	Est.	Est.
Band 1	Low	15.16**	15.98**	10.68**	6.88**
	Medium	17.17**	23.22**	23.45**	15.79**
	High	26.32**	22.77**	26.09**	19.81**
Band 2	Low	-0.11	3.24**	-2.00	-1.29
	Medium	0.50	4.31**	2.24	-1.14
	High	5.51**	8.85**	7.79**	3.29*
Band 3	Low	-3.17**	-3.78**	-4.98**	-3.33**
	Medium	-5.47**	-3.45**	-5.26**	-5.40**
	High	-6.67**	-7.84**	-6.43**	-6.15**
Band 4	Low	-3.64**	-4.42**	-0.16	-1.00
	Medium	-5.82**	-8.59**	-7.97**	-6.49**
	High	-9.20**	-3.34	-8.50**	-6.90**
Band 5	Low	-8.66**	-11.34**	-3.50*	-1.80
	Medium	-5.76**	-15.86**	-12.92**	-3.82*
	High	-15.12**	-18.22**	-18.33**	-8.98**

* Denotes statistical significance at the p = 0.05 level.
** Denotes statistical significance at the p = 0.01 level.

TABLE 9. ESTIMATED DIFFERENCE IN BASELINE SHIFT BETWEEN EACH LEVEL OF ATROPINE DOSE AND SALINE CONTROL DOSE FOR PERCENT POWER IN ALL 5 FREQUENCY BANDS BASED ON MODEL (1) IN CHANNEL 2

	Dose Level	30 min. post-dose	90 min. post-dose	300 min. post-dose	600 min. post-dose
		Est.	Est.	Est.	Est.
Band 1	Low	6.88**	8.94**	4.52*	1.21
	Medium	11.69**	13.71**	12.08**	7.10**
	High	21.38**	21.44**	22.72**	13.05**
Band 2	Low	2.08	3.03*	0.00	-5.21**
	Medium	0.84	5.91**	1.64	-4.18**
	High	5.59**	8.50**	7.24**	3.68**
Band 3	Low	-4.22**	-4.96**	-5.30**	-2.60*
	Medium	-5.73**	-3.50**	-4.34**	-3.87**
	High	-5.55**	-5.51**	-4.78**	-1.01
Band 4	Low	-1.27	-3.64**	-0.56	2.38*
	Medium	-3.99**	-6.93**	-7.01**	-2.37*
	High	-8.74**	-7.60**	-10.73**	-7.46**
Band 5	Low	-3.75*	-3.57**	1.35	3.61**
	Medium	-2.54	-9.50**	-2.72*	2.20
	High	-12.42**	-14.94**	-14.17**	-7.98**

* Denotes statistical significance at the p = 0.05 level.
** Denotes statistical significance at the p = 0.01 level.

TABLE 10. ESTIMATED DIFFERENCE IN BASELINE SHIFT BETWEEN EACH LEVEL OF ATROPINE DOSE AND SALINE CONTROL DOSE FOR PERCENT POWER IN ALL 5 FREQUENCY BANDS BASED ON MODEL (1) IN CHANNEL 3

	Dose Level	30 min. post-dose	90 min. post-dose	300 min. post-dose	600 min. post-dose
		Est.	Est.	Est.	Est.
Band 1	Low	8.61**	8.90**	1.32	-3.27
	Medium	9.57**	18.44**	14.18**	7.16**
	High	18.62**	31.58**	22.67**	13.31**
Band 2	Low	-0.75	1.22	-3.70**	-3.02*
	Medium	1.24	5.68**	1.65	-1.02
	High	3.22*	3.69*	1.99	0.52
Band 3	Low	-0.89	-1.03	-4.69**	-1.93
	Medium	-2.37	-3.76**	-6.83**	-5.62**
	High	-1.56	-5.12**	-6.31**	-2.86*
Band 4	Low	-0.69	-0.30	3.58**	2.82**
	Medium	-2.97*	-7.02**	-4.19**	-3.24**
	High	-6.98**	-6.44**	-5.57**	-4.55**
Band 5	Low	-6.48**	-8.93**	3.67*	4.88**
	Medium	-5.06**	-13.67**	-5.18**	1.72
	High	-12.74**	-21.97**	-12.02**	-5.71**

* Denotes statistical significance at the p = 0.05 level.
** Denotes statistical significance at the p = 0.01 level.

TABLE 11. ESTIMATED DIFFERENCE IN BASELINE SHIFT BETWEEN EACH LEVEL OF ATROPINE DOSE AND SALINE CONTROL DOSE FOR PERCENT POWER IN ALL 5 FREQUENCY BANDS BASED ON MODEL (1) IN CHANNEL 4

	Dose Level	30 min. post-dose	90 min. post-dose	300 min. post-dose	600 min. post-dose
		Est.	Est.	Est.	Est.
Band 1	Low	7.64**	7.11**	1.57	-1.75
	Medium	8.23**	19.14**	17.14**	7.91**
	High	16.71**	22.61**	21.84**	11.92**
Band 2	Low	-0.48	-0.25	-2.50	-4.21**
	Medium	0.03	3.46*	1.43	-1.45
	High	4.37**	1.49	4.93**	0.51
Band 3	Low	-2.77*	-1.85	-4.48**	-3.27**
	Medium	-4.47**	-4.06**	-7.89**	-7.44**
	High	-5.31**	-6.97**	-7.59**	-5.66**
Band 4	Low	-2.19	-1.27	2.44*	0.64
	Medium	-4.34**	-8.67**	-6.31**	-5.58**
	High	-7.21**	-4.47**	-7.70**	-5.56**
Band 5	Low	-2.52	-4.02**	2.96*	7.81**
	Medium	0.87	-10.28**	-4.81**	5.39**
	High	-7.77**	-10.70**	-10.53**	-0.41

* Denotes statistical significance at the p = 0.05 level.
** Denotes statistical significance at the p = 0.01 level.

Model (2) was fit to the percent power data in the first frequency band through least-squares analysis of variance. The model was fit separately for all six animals, and for each channel for each animal.

Prior to fitting Model (2), a correlation analysis was conducted to assess the impact of potential correlation of data within a time period. Correlation coefficients were minimal (generally less than 0.25) and therefore were not compensated for in Model (2).

As before, F-tests were implemented in the analysis of variance to determine whether effects of atropine dosing and time period were statistically significant, and whether the extent of the atropine effect significantly differed between time periods. In addition, linear contrasts of model parameters were performed to determine:

- Those post-dosing periods of time with significant shifts in percent power from baseline for a given dosing experiment,
- The significance of the difference in shift from baseline between the atropine and saline control experiments.

Tables 12 to 21 summarize the statistical analyses of the percent power data in the first frequency band for the six animals (038D, 122X, 4E5, D275, D345 and E109). Their interpretation is similar to that for Tables 2 and 3. Note, however, that no table exists to illustrate differences in baseline shift from the saline control for animals D345 and E109 due to lack of data across the dose groups.

Well over 100 statistical tests are represented in Tables 2 to 21. If conclusions are made at the 0.05 significance level on 100 statistical tests, we would expect statistical significance to be found in five of these tests due simply to the randomness present in the data. Thus, an isolated result for a single channel, time period, or atropine dose must be interpreted with caution.

TABLE 12. ESTIMATED DIFFERENCE FROM BASELINE IN PERCENT POWER IN THE FIRST FREQUENCY BAND, WITH STANDARD ERROR AND LEVEL OF SIGNIFICANCE FROM ZERO, FOR ANIMAL 038D

	Dose Level	30 min. post-dose			90 min. post-dose			300 min. post-dose			600 min. post-dose		
		Est.	S.E.	p	Est.	S.E.	p	Est.	S.E.	p	Est.	S.E.	p
Channel 1	Saline	1.19	3.01	0.693	-5.96	3.01	0.049	-9.96	3.01	0.001	-2.22	3.01	0.463
	High				22.30	3.01	0.000	27.22	3.01	0.000	22.03	3.01	0.000
Channel 2	Saline	5.13	2.52	0.043	-3.03	2.52	0.230	-6.60	2.52	0.009	4.81	2.52	0.057
	High				21.82	2.52	0.000	23.43	2.52	0.000	16.59	2.52	0.000
Channel 3	Saline	0.82	2.71	0.762	-5.69	2.71	0.036	-3.85	2.71	0.157	4.36	2.71	0.109
	High				31.28	2.71	0.000	20.16	2.71	0.000	19.21	2.71	0.000
Channel 4	Saline	1.83	2.87	0.524	-2.46	2.87	0.392	-4.47	2.87	0.120	7.29	2.87	0.012
	High				25.30	2.87	0.000	23.04	2.87	0.000	17.39	2.87	0.000

1. "Est." is the estimated difference from the baseline (pre-dose) time period in percent power in the first frequency band for the given dose level/time period combination. "S.E." is the estimated standard error in this estimate. "p" is the significance level for testing whether this difference from baseline is significantly different from zero.

2. "Saline" is 0.0 mg/kg atropine, "High" is 0.4 mg/kg atropine. Data at the baseline time period were not available for this animal at the 0.1 mg/kg atropine ("Low") and 0.2 mg/kg atropine ("Medium") dose levels. Data also were not available at time period/dose combinations where no results are provided in the table.

3. The order of dosing for this animal was as follows: Low, High, Medium, Saline.

TABLE 13. ESTIMATED DIFFERENCE FROM BASELINE IN PERCENT POWER IN THE FIRST FREQUENCY BAND, WITH STANDARD ERROR AND LEVEL OF SIGNIFICANCE FROM ZERO, FOR ANIMAL 122X

	Dose Level	30 min. post-dose			90 min. post-dose			300 min. post-dose			600 min. post-dose		
		Est.	S.E.	p	Est.	S.E.	p	Est.	S.E.	p	Est.	S.E.	p
Channel 1	Saline				0.46	3.34	0.892	0.88	3.34	0.793	1.89	3.34	0.573
	Low				8.95	3.34	0.008	7.91	3.34	0.019	12.39	3.34	0.000
	Medium				0.90	3.34	0.787	8.23	3.34	0.014	8.56	3.34	0.011
	High	12.57	3.34	0.000				16.73	3.34	0.000	19.88	3.34	0.000
Channel 2	Saline				2.63	3.02	0.385	3.95	3.02	0.192	3.12	3.02	0.302
	Low				3.70	3.02	0.222	5.04	3.02	0.096	8.85	3.02	0.004
	Medium				7.19	3.02	0.018	4.84	3.02	0.110	6.54	3.02	0.031
	High	17.50	3.02	0.000				19.83	3.02	0.000	6.20	3.02	0.041
Channel 3	Saline				5.71	2.93	0.052	5.88	2.93	0.046	3.99	2.93	0.174
	Low				7.64	2.93	0.009	0.50	2.93	0.864	6.85	2.93	0.020
	Medium				7.56	2.93	0.010	7.32	2.93	0.013	5.39	2.93	0.066
	High	10.03	2.93	0.001				16.40	2.93	0.000	12.36	2.93	0.000
Channel 4	Saline				3.93	2.74	0.153	5.90	2.74	0.032	7.60	2.74	0.006
	Low				11.21	2.74	0.000	2.41	2.74	0.380	8.33	2.74	0.003
	Medium				6.28	2.74	0.023	10.93	2.74	0.000	6.49	2.74	0.018
	High	10.29	2.74	0.000				15.45	2.74	0.000	13.27	2.74	0.000

1. "Est." is the estimated difference from the baseline (pre-dose) time period in percent power in the first frequency band for the given dose level/time period combination. "S.E." is the estimated standard error in this estimate. "p" is the significance level for testing whether this difference from baseline is significantly different from zero.

2. "Saline" is 0.0 mg/kg atropine, "Low" is 0.1 mg/kg atropine, "Medium" is 0.2 mg/kg atropine, "High" is 0.4 mg/kg atropine. Data were not available at time period/dose combinations where no results are provided in the table.

3. The order of dosing for this animal was as follows: High, Medium, Low, Saline.

TABLE 14. ESTIMATED DIFFERENCE FROM BASELINE IN PERCENT POWER IN THE FIRST FREQUENCY BAND, WITH STANDARD ERROR AND LEVEL OF SIGNIFICANCE FROM ZERO, FOR ANIMAL 4E5

	Dose Level	30 min. post-dose			90 min. post-dose			300 min. post-dose			600 min. post-dose		
		Est.	S.E.	p	Est.	S.E.	p	Est.	S.E.	p	Est.	S.E.	p
Channel 1	Saline	-12.03	3.29	0.000	-10.49	3.29	0.002	-14.88	3.29	0.000	-7.18	3.29	0.030
	Low	18.25	3.29	0.000	16.84	3.29	0.000	13.83	3.29	0.000	25.45	3.29	0.000
	Medium										15.04	3.29	0.000
Channel 2	Saline	-4.32	2.49	0.085	-6.28	2.49	0.012	-6.43	2.49	0.010	-0.16	2.49	0.948
	Low	11.04	2.49	0.000	11.72	2.49	0.000	6.43	2.49	0.010	9.25	2.49	0.000
	Medium										9.09	2.49	0.000
Channel 3	Saline	-10.11	2.40	0.000	-10.77	2.40	0.000	-12.88	2.40	0.000	-6.58	2.40	0.007
	Low	10.36	2.40	0.000	11.59	2.40	0.000	5.11	2.40	0.034	1.94	2.40	0.421
	Medium										4.98	2.40	0.039
Channel 4	Saline	-7.39	2.62	0.005	-5.85	2.62	0.027	-9.76	2.62	0.000	-3.78	2.62	0.151
	Low	14.26	2.62	0.000	13.13	2.62	0.000	11.62	2.62	0.000	18.20	2.62	0.000
	Medium										10.06	2.62	0.000

1. "Est." is the estimated difference from the baseline (pre-dose) time period in percent power in the first frequency band for the given dose level/time period combination. "S.E." is the estimated standard error in this estimate. "p" is the significance level for testing whether this difference from baseline is significantly different from zero.

2. "Saline" is 0.0 mg/kg atropine, "Low" is 0.1 mg/kg atropine, "Medium" is 0.2 mg/kg atropine. Data at the baseline time period were not available for this animal at the 0.4 mg/kg atropine ("High") dose level. Data also were not available at time period/dose combinations where no results are provided in the table.

3. The order of dosing for this animal was as follows: Medium, Low, High, Saline.

TABLE 15. ESTIMATED DIFFERENCE FROM BASELINE IN PERCENT POWER IN THE FIRST FREQUENCY BAND, WITH STANDARD ERROR AND LEVEL OF SIGNIFICANCE FROM ZERO, FOR ANIMAL D275

	Dose Level	30 min. post-dose			90 min. post-dose			300 min. post-dose			600 min. post-dose		
		Est.	S.E.	p	Est.	S.E.	p	Est.	S.E.	p	Est.	S.E.	p
Channel 1	Saline	3.93	3.91	0.316	6.15	3.91	0.117	1.53	3.91	0.697	10.64	3.91	0.007
	Low	15.86	3.91	0.000	11.09	3.91	0.005	-8.71	3.91	0.026	-7.45	3.91	0.057
	Medium	29.68	3.91	0.000	19.75	3.91	0.000	22.80	3.91	0.000	14.51	3.91	0.000
	High	34.29	3.91	0.000				13.08	3.91	0.001	10.16	3.91	0.010
Channel 2	Saline	3.39	3.32	0.307	2.63	3.32	0.428	3.16	3.32	0.341	5.76	3.32	0.083
	Low	11.44	3.32	0.001	7.96	3.32	0.017	-3.95	3.32	0.234	-2.55	3.32	0.443
	Medium	22.60	3.32	0.000	10.91	3.32	0.001	18.12	3.32	0.000	11.01	3.32	0.001
	High	27.22	3.32	0.000				17.13	3.32	0.000	20.03	3.32	0.000
Channel 3	Saline	7.77	3.10	0.013	5.54	3.10	0.075	1.05	3.10	0.735	8.06	3.10	0.010
	Low	13.32	3.10	0.000	10.41	3.10	0.001	-12.02	3.10	0.000	-11.34	3.10	0.000
	Medium	15.34	3.10	0.000	16.26	3.10	0.000	21.56	3.10	0.000	10.06	3.10	0.001
	High	29.08	3.10	0.000				23.07	3.10	0.000	11.87	3.10	0.000
Channel 4	Saline	6.33	3.16	0.046	6.72	3.16	0.034	-0.84	3.16	0.792	1.36	3.16	0.667
	Low	7.44	3.16	0.019	5.99	3.16	0.059	-17.03	3.16	0.000	-14.37	3.16	0.000
	Medium	17.30	3.16	0.000	19.74	3.16	0.000	24.96	3.16	0.000	13.77	3.16	0.000
	High	28.06	3.16	0.000				22.72	3.16	0.000	15.29	3.16	0.000

1. "Est." is the estimated difference from the baseline (pre-dose) time period in percent power in the first frequency band for the given dose level/time period combination. "S.E." is the estimated standard error in this estimate. "p" is the significance level for testing whether this difference from baseline is significantly different from zero.

2. "Saline" is 0.0 mg/kg atropine, "Low" is 0.1 mg/kg atropine, "Medium" is 0.2 mg/kg atropine and "High" is 0.4 mg/kg atropine. Data were not available at time period/dose combinations where no results are provided in the table.

3. The order of dosing for this animal was as follows: High, Low, Medium, Saline.

TABLE 16. ESTIMATED DIFFERENCE FROM BASELINE IN PERCENT POWER IN THE FIRST FREQUENCY BAND, WITH STANDARD ERROR AND LEVEL OF SIGNIFICANCE FROM ZERO, FOR ANIMAL D345

	Dose Level	30 min. post-dose			90 min. post-dose			300 min. post-dose			600 min. post-dose		
		Est.	S.E.	p	Est.	S.E.	p	Est.	S.E.	p	Est.	S.E.	p
Channel 1	Saline	5.12	3.16	0.106	-13.96	3.16	0.000	-16.00	3.16	0.000	-14.65	3.16	0.000
Channel 2	Saline	3.12	2.74	0.255	-6.57	2.74	0.017	-7.57	2.74	0.006	-7.08	2.74	0.010
Channel 3	Saline	1.50	2.43	0.538	-5.40	2.43	0.027	-5.63	2.43	0.021	-4.66	2.43	0.056
Channel 4	Saline	8.95	2.28	0.000	3.08	2.28	0.178	1.94	2.28	0.396	1.81	2.28	0.429

1. "Est." is the estimated difference from the baseline (pre-dose) time period in percent power in the first frequency band for the given dose level/time period combination. "S.E." is the estimated standard error in this estimate. "p" is the significance level for testing whether this difference from baseline is significantly different from zero.

2. "Saline" is 0.0 mg/kg atropine. Data at the baseline time period were not available for this animal at the 0.1 mg/kg atropine ("Low") and 0.2 mg/kg atropine ("Medium") dose levels, and were not available at post-dose time periods at the 0.4 mg/kg atropine ("High") dose level.

3. The order of dosing for this animal was as follows: Low, Medium, High, Saline.

TABLE 17. ESTIMATED DIFFERENCE FROM BASELINE IN PERCENT POWER IN THE FIRST FREQUENCY BAND, WITH STANDARD ERROR AND LEVEL OF SIGNIFICANCE FROM ZERO, FOR ANIMAL E109

	Dose Level	30 min. post-dose			90 min. post-dose			300 min. post-dose			600 min. post-dose		
		Est.	S.E.	p	Est.	S.E.	p	Est.	S.E.	p	Est.	S.E.	p
Channel 1	Low	7.87	2.92	0.007	7.24	2.92	0.013	-10.95	2.92	0.000	-1.40	2.92	0.632
	Medium	11.51	2.92	0.000	24.60	2.92	0.000	27.39	2.92	0.000	19.12	2.92	0.000
Channel 2	Low	2.77	2.97	0.352	5.35	2.97	0.073	-2.94	2.97	0.324	-3.01	2.97	0.311
	Medium	7.71	2.97	0.010	13.82	2.97	0.000	7.24	2.97	0.015	10.98	2.97	0.000
Channel 3	Low	4.01	2.52	0.113	-3.91	2.52	0.122	-1.46	2.52	0.563	-6.43	2.52	0.011
	Medium	12.57	2.52	0.000	16.54	2.52	0.000	13.31	2.52	0.000	15.09	2.52	0.000
Channel 4	Low	7.61	2.67	0.005	1.51	2.67	0.571	-2.01	2.67	0.452	-2.25	2.67	0.400
	Medium	11.92	2.67	0.000	19.83	2.67	0.000	21.24	2.67	0.000	21.09	2.67	0.000

1. "Est." is the estimated difference from the baseline (pre-dose) time period in percent power in the first frequency band for the given dose level/time period combination. "S.E." is the estimated standard error in this estimate. "p" is the significance level for testing whether this difference from baseline is significantly different from zero.

2. "Low" is 0.1 mg/kg atropine, "Medium" is 0.2 mg/kg atropine. Data at the baseline time period were not available for this animal at the 0.0 mg/kg atropine ("Saline") dose level and were not available in post-dose time periods at the 0.4 mg/kg atropine ("High") level.

3. The order of dosing for this animal was as follows: Medium, High, Low, Saline.

TABLE 18. ESTIMATED DIFFERENCE IN BASELINE SHIFT BETWEEN EACH DOSE LEVEL AND SALINE CONTROL DOSE FOR PERCENT POWER IN THE FIRST FREQUENCY BAND, WITH STANDARD ERROR AND LEVEL OF SIGNIFICANCE FROM ZERO, FOR ANIMAL 038D

	Dose Level	30 min. post-dose			90 min. post-dose			300 min. post-dose			600 min. post-dose		
		Est.	S.E.	p	Est.	S.E.	p	Est.	S.E.	p	Est.	S.E.	p
Channel 1	High				28.26	4.26	0.000	37.17	4.26	0.000	24.25	4.26	0.000
Channel 2	High				24.85	3.56	0.000	30.03	3.56	0.000	11.78	3.56	0.001
Channel 3	High				36.98	3.83	0.000	24.01	3.83	0.000	14.86	3.83	0.000
Channel 4	High				27.76	4.06	0.000	27.52	4.06	0.000	10.09	4.06	0.013

1. For percent power in the first frequency band, "Est." is the estimated difference in the baseline shifts (Table 2) between the given dose level and the saline control dose. "S.E." is the estimated standard error in this estimate. "p" is the significance level for testing whether this difference from the saline control dose is significantly different from zero.

2. "High" is 0.4 mg/kg atropine. Data at the baseline time period were not available for this animal at the 0.1 mg/kg atropine ("Low") and 0.2 mg/kg atropine ("Medium") dose levels. Data also were not available at the high dose level at the 30 min. time period.

3. The order of dosing for this animal was as follows: Low, High, Medium, Saline.

TABLE 19. ESTIMATED DIFFERENCE IN BASELINE SHIFT BETWEEN EACH DOSE LEVEL AND SALINE CONTROL DOSE FOR PERCENT POWER IN THE FIRST FREQUENCY BAND, WITH STANDARD ERROR AND LEVEL OF SIGNIFICANCE FROM ZERO, FOR ANIMAL 122X

	Dose Level	30 min. post-dose			90-min. post-dose			300 min. post-dose			600 min. post-dose		
		Est.	S.E.	p	Est.	S.E.	p	Est.	S.E.	p	Est.	S.E.	p
Channel 1	Low				8.49	4.73	0.073	7.03	4.73	0.138	10.50	4.73	0.027
	Medium				0.45	4.73	0.925	7.36	4.73	0.120	6.68	4.73	0.159
	High							15.85	4.73	0.001	18.00	4.73	0.000
Channel 2	Low				1.07	4.28	0.803	1.09	4.28	0.798	5.73	4.28	0.181
	Medium				4.56	4.28	0.287	0.89	4.28	0.835	3.42	4.28	0.425
	High							15.88	4.28	0.000	3.08	4.28	0.471
Channel 3	Low				1.93	4.14	0.642	-5.37	4.14	0.195	2.87	4.14	0.490
	Medium				1.85	4.14	0.656	1.44	4.14	0.728	1.41	4.14	0.734
	High							10.53	4.14	0.011	8.37	4.14	0.044
Channel 4	Low				7.28	3.88	0.061	-3.49	3.88	0.369	0.73	3.88	0.851
	Medium				2.35	3.88	0.545	5.03	3.88	0.196	-1.11	3.88	0.775
	High							9.55	3.88	0.014	5.67	3.88	0.145

1. For percent power in the first frequency band, "Est." is the estimated difference in the baseline shifts (Table 3) between the given dose level and the saline control dose. "S.E." is the estimated standard error in this estimate. "p" is the significance level for testing whether this difference from the saline control dose is significantly different from zero.

2. "Low" is 0.1 mg/kg atropine, "Medium" is 0.2 mg/kg atropine, "High" is 0.4 mg/kg atropine. Data at the saline control level were not available for this animal at the 30 min. time period, and data at the high dose level were not available at the 90 min. time period.

3. The order of dosing for this animal was as follows: High, Medium, Low, Saline.

TABLE 20. ESTIMATED DIFFERENCE IN BASELINE SHIFT BETWEEN EACH DOSE LEVEL AND SALINE CONTROL DOSE FOR PERCENT POWER IN THE FIRST FREQUENCY BAND, WITH STANDARD ERROR AND LEVEL OF SIGNIFICANCE FROM ZERO, FOR ANIMAL 4E5

	Dose Level	30 min. post-dose			90 min. post-dose			300 min. post-dose			600 min. post-dose		
		Est.	S.E.	p	Est.	S.E.	p	Est.	S.E.	p	Est.	S.E.	p
Channel 1	Low	30.28	4.65	0.000	27.33	4.65	0.000	28.71	4.65	0.000	32.64	4.65	0.000
	Medium										22.22	4.65	0.000
Channel 2	Low	15.36	3.53	0.000	18.00	3.53	0.000	12.86	3.53	0.000	9.41	3.53	0.008
	Medium										9.25	3.53	0.009
Channel 3	Low	20.47	3.40	0.000	22.36	3.40	0.000	17.99	3.40	0.000	8.51	3.40	0.013
	Medium										11.56	3.40	0.001
Channel 4	Low	21.65	3.71	0.000	18.98	3.71	0.000	21.38	3.71	0.000	21.98	3.71	0.000
	Medium										13.83	3.71	0.000

1. For percent power in the first frequency band, "Est." is the estimated difference in the baseline shifts (Table 4) between the given dose level and the saline control dose. "S.E." is the estimated standard error in this estimate. "p" is the significance level for testing whether this difference from the saline control dose is significantly different from zero.

2. "Low" is 0.1 mg/kg atropine, "Medium" is 0.2 mg/kg atropine. Data at the baseline time period were not available for this animal at the 0.4 mg/kg atropine ("High") dose level. Data at the medium dose level were not available at the 30, 90, or 600 min. time periods.

3. The order of dosing for this animal was as follows: Medium, Low, High, Saline.

TABLE 21. ESTIMATED DIFFERENCE IN BASELINE SHIFT BETWEEN EACH DOSE LEVEL AND SALINE CONTROL DOSE FOR PERCENT POWER IN THE FIRST FREQUENCY BAND, WITH STANDARD ERROR AND LEVEL OF SIGNIFICANCE FROM ZERO, FOR ANIMAL D275

	Dose Level	30 min. post-dose			90 min. post-dose			300 min. post-dose			600 min. post-dose		
		Est.	S.E.	p	Est.	S.E.	p	Est.	S.E.	p	Est.	S.E.	p
Channel 1	Low	11.93	5.53	0.031	4.94	5.53	0.372	-10.23	5.53	0.065	-18.09	5.53	0.001
	Medium	25.76	5.53	0.000	13.61	5.53	0.014	21.28	5.53	0.000	3.87	5.53	0.484
	High	30.37	5.53	0.000				11.55	5.53	0.037	-0.48	5.53	0.931
Channel 2	Low	8.05	4.69	0.086	5.33	4.69	0.256	-7.11	4.69	0.130	-8.31	4.69	0.077
	Medium	19.21	4.69	0.000	8.28	4.69	0.078	14.97	4.69	0.002	5.25	4.69	0.263
	High	23.83	4.69	0.000				13.97	4.69	0.003	14.27	4.69	0.002
Channel 3	Low	5.55	4.39	0.206	4.87	4.39	0.268	-13.08	4.39	0.003	-19.40	4.39	0.000
	Medium	7.57	4.39	0.085	10.72	4.39	0.015	20.50	4.39	0.000	2.00	4.39	0.649
	High	21.31	4.39	0.000				22.01	4.39	0.000	3.81	4.39	0.386
Channel 4	Low	1.11	4.47	0.805	-0.73	4.47	0.870	-16.19	4.47	0.000	-15.73	4.47	0.001
	Medium	10.97	4.47	0.014	13.02	4.47	0.004	25.80	4.47	0.000	12.41	4.47	0.006
	High	21.73	4.47	0.000				23.56	4.47	0.000	13.93	4.47	0.002

1. For percent power in the first frequency band, "Est." is the estimated difference in the baseline shifts (Table 5) between the given dose level and the saline control dose. "S.E." is the estimated standard error in this estimate. "p" is the significance level for testing whether this difference from the saline control dose is significantly different from zero.

2. "Low" is 0.1 mg/kg atropine, "Medium" is 0.2 mg/kg atropine, "High" is 0.4 mg/kg atropine. Data at the high dose level were not available at the 90 min. time period.

3. The order of dosing for this animal was as follows: High, Low, Medium, Saline.

4.0 RESULTS AND CONCLUSIONS

The following conclusions are based on the data summaries displayed in the appendices and the results of the statistical analyses that were presented in the previous section of this report.

4.1 RESULTS BASED ON MODEL (1) INTRA-ANIMAL ANALYSIS

Estimates from Model (1), displayed in Tables 4 to 7, demonstrate shifts from baseline (0 minute time point) averaged across all animals for each combination of frequency band, channel, dose, and time period. Positive average shifts from baseline indicate higher percent power readings in a frequency band for the post-dosing period relative to baseline. Negative average shifts from baseline represent a decline in the percent power relative to baseline. The following conclusions are based on the results of the statistical analysis presented in Tables 4 to 7:

- Average baseline shifts in percent power in the first frequency band were statistically significant and positive for the atropine 0.4 and 0.2 mg/kg dose experiments, at all four post-dosing periods of time, and all four channels. The average baseline shifts in the first frequency band were also statistically significant and positive at 30 and 90 minutes for the 0.1 mg/kg atropine dose experiments, in all four channels. In all cases, these shifts were significant even at the 0.01 level, but the magnitudes of these shifts appear to diminish with time since dosing. There was no noticeable trend in the average shifts from baseline at 300 and 600 minutes for the 0.1 mg/kg atropine dose experiments.

- Average baseline shifts in percent power in the fifth frequency band were statistically significant and negative for the 0.4 mg/kg atropine dose experiments, at all four post-dosing periods of time, and all four channels. The average baseline shifts in the fifth frequency band were mostly negative and significant at 30 and 90 minutes for the 0.1 and 0.2 mg/kg atropine dose experiments, in all four channels. The magnitude of baseline shifts in the fifth frequency band also appear to diminish with respect to time after dosing.

- For the saline dose experiments, shifts in percent power from baseline were sometimes positive, sometimes negative, and sometimes significantly different from zero in all five frequency bands and all four channels. The magnitudes of the shifts from baseline observed in the saline control experiments were generally lower than in the atropine dose experiments.

- Shifts in percent power from baseline for dosing experiments were generally positive and large in magnitude for the first frequency band, positive and of smaller magnitude in band 2, negative and of small magnitude in bands 3 and 4, and negative and large in magnitude in the fifth frequency band. This pattern is demonstrated in Figures 1 to 16.

- The effect of atropine on baseline shifts in percent power is most clearly seen by comparing the effect of the atropine doses on the first and fifth frequency bands over time. This comparison supports the conclusion that:

  (1) Atropine produces dose-related shifts in percent power.

  (2) Positive shifts noted in the first (primarily) and second frequency bands are accompanied by negative shifts in bands 3 through 5 (5 primarily).

  (3) The magnitude of this dose response relationship diminishes with time after dosing.

4.2 RESULTS BASED ON MODEL (2) INTER-ANIMAL ANALYSIS

Estimates from Model (2), displayed in tables 12 to 17, demonstrate average shifts from baseline in the first frequency band specific to each animal for each combination of channel, dose, and time period. These shifts from baseline have similar interpretation to those in the previous section, the difference being that Model (2) is animal-specific, and was applied only to measurements of percent power occurring in the first frequency band. Conclusions from fitting Model (2), as presented in Tables 12 to 17, are as follows:

- Baseline shifts in the first frequency band estimated individually for each animal using Model (2) were always positive for the 0.4 and 0.2 mg/kg atropine dose experiments, and were sometimes statistically significant

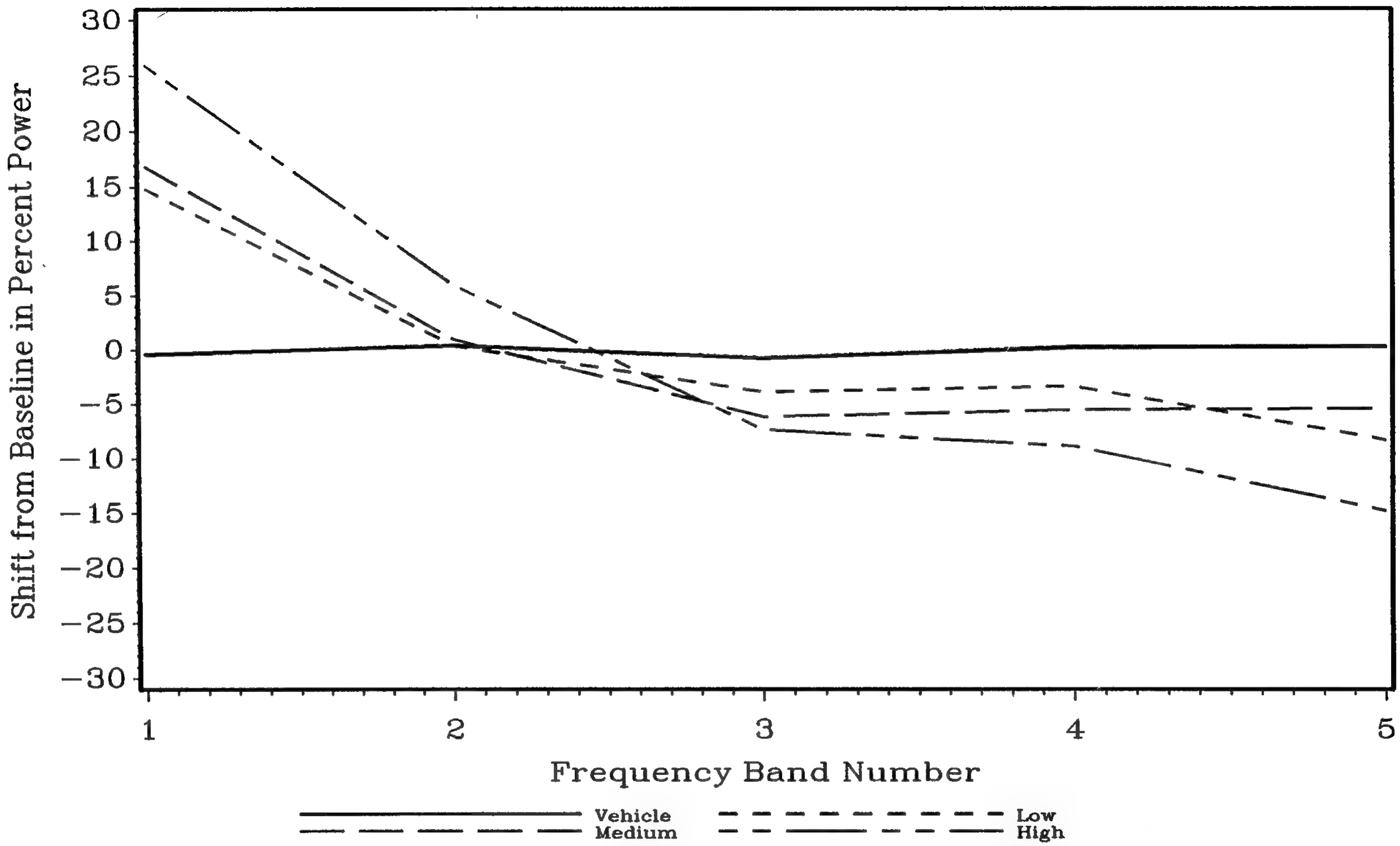

FIGURE 1. PLOT OF SHIFT FROM BASELINE IN PERCENT POWER VERSUS FREQUENCY BAND FOR CHANNEL 1 AT 30 MINUTES

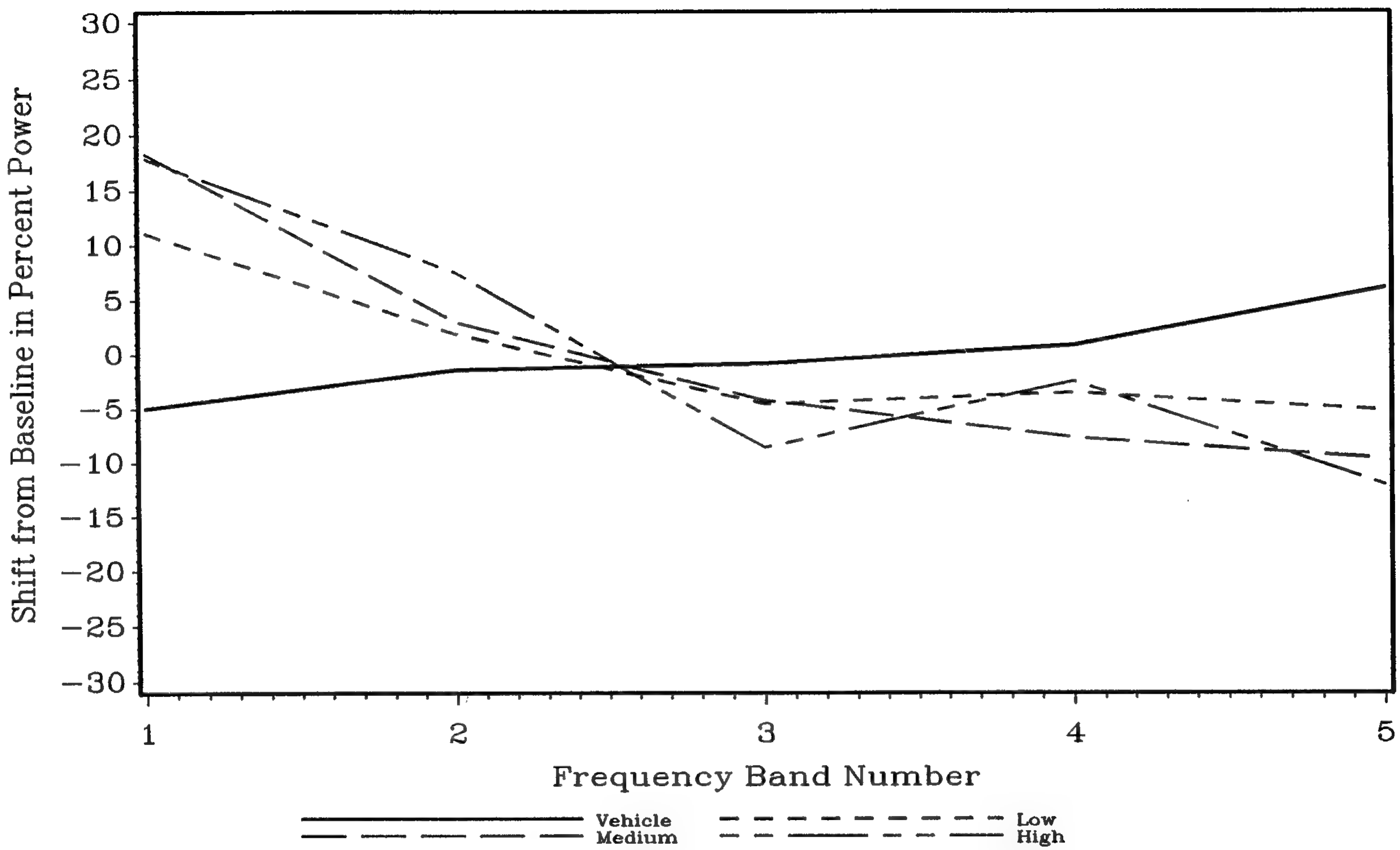

FIGURE 2. PLOT OF SHIFT FROM BASELINE IN PERCENT POWER VERSUS FREQUENCY BAND FOR CHANNEL 1 AT 90 MINUTES

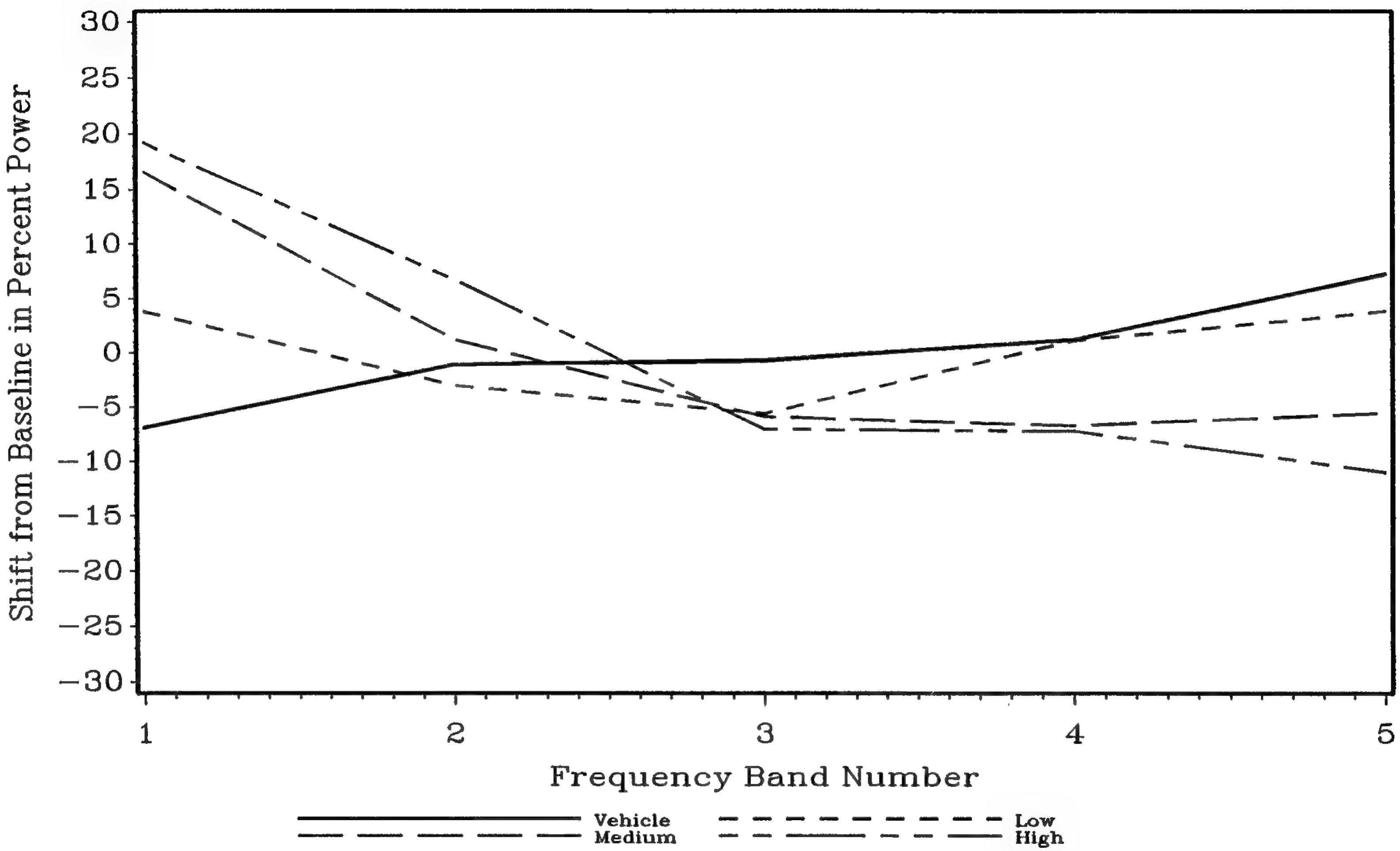

FIGURE 3. PLOT OF SHIFT FROM BASELINE IN PERCENT POWER VERSUS FREQUENCY BAND FOR CHANNEL 1 AT 300 MINUTES

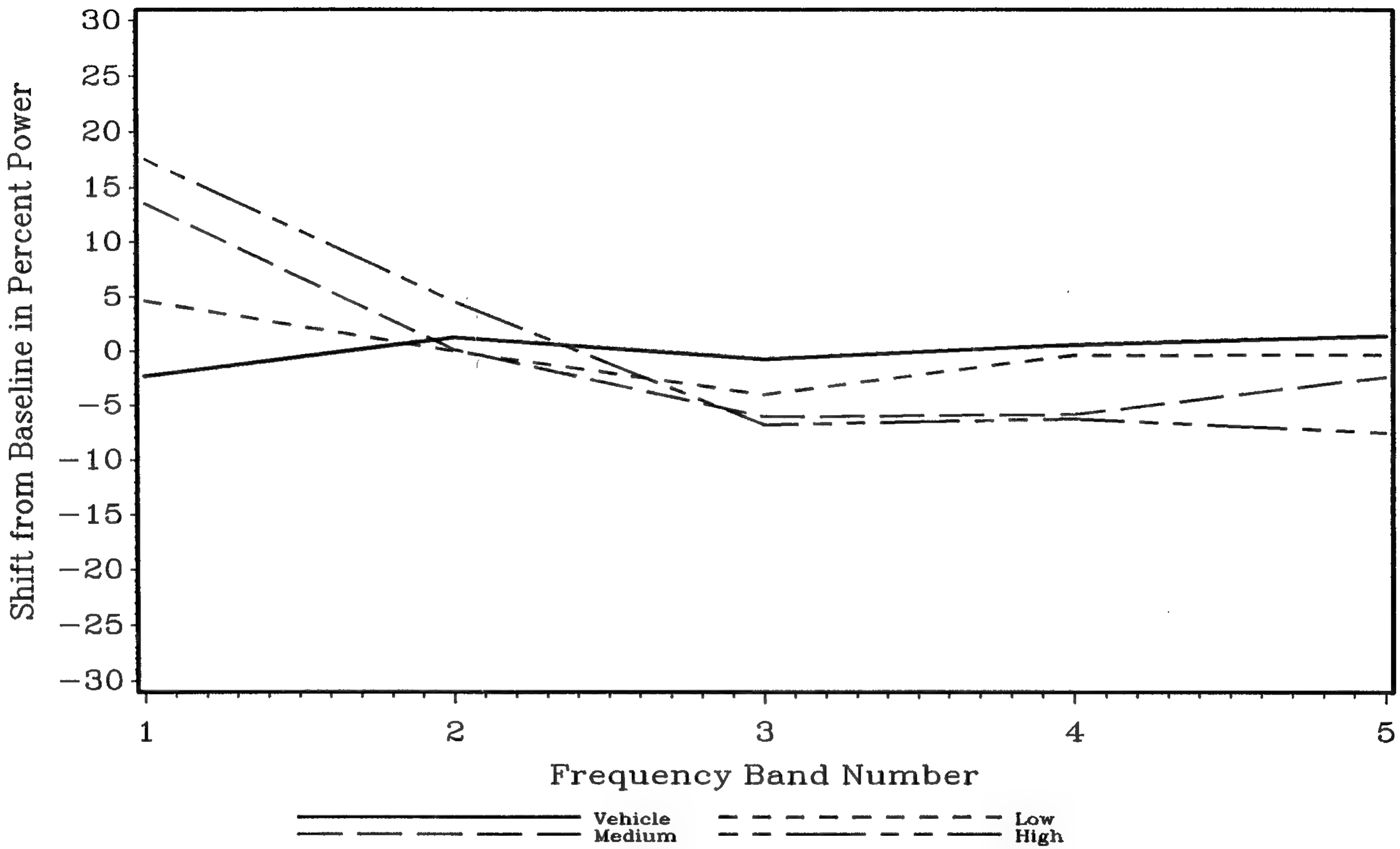

FIGURE 4. PLOT OF SHIFT FROM BASELINE IN PERCENT POWER VERSUS FREQUENCY BAND FOR CHANNEL 1 AT 600 MINUTES

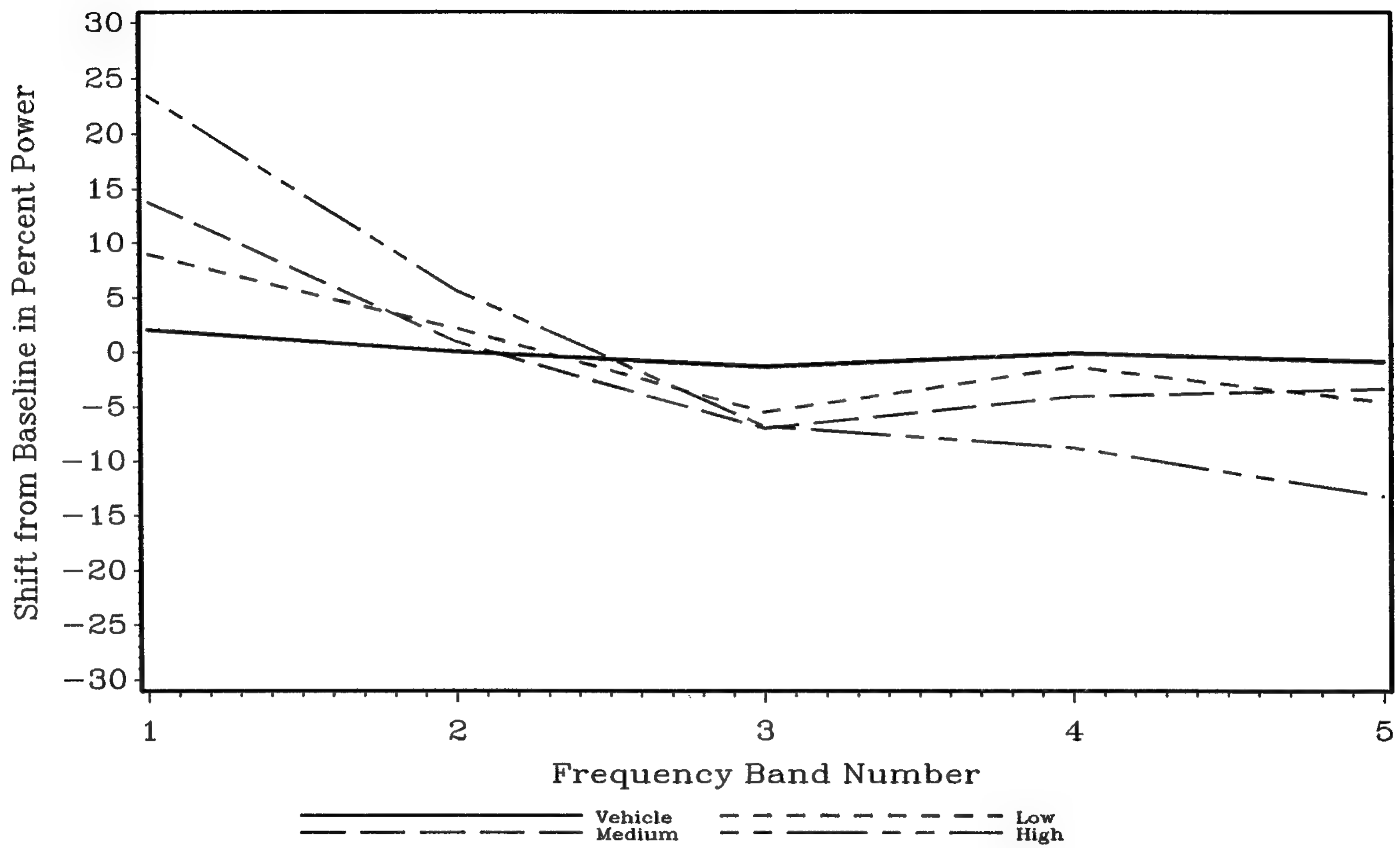

FIGURE 5. PLOT OF SHIFT FROM BASELINE IN PERCENT POWER VERSUS FREQUENCY BAND FOR CHANNEL 2 AT 30 MINUTES

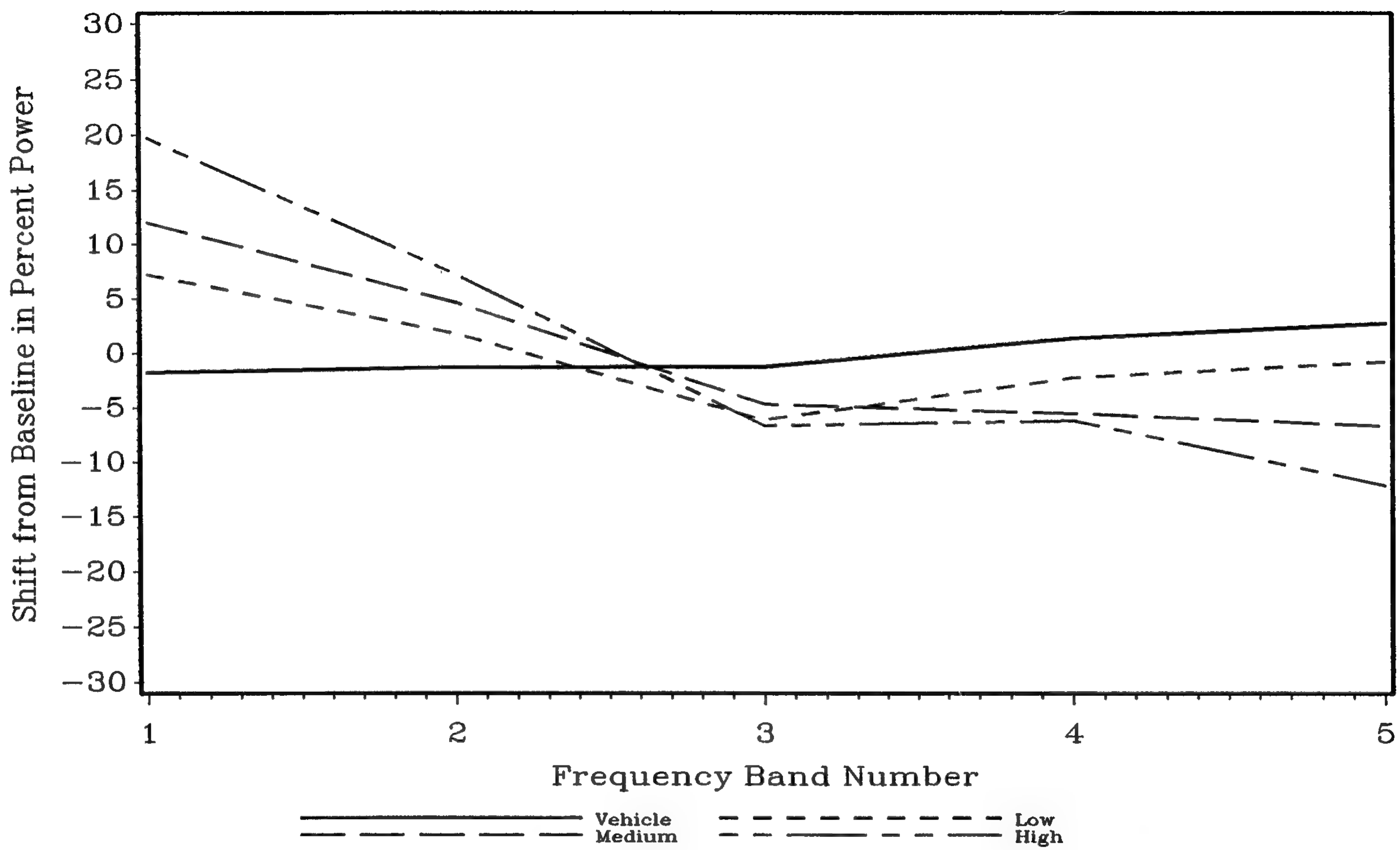

FIGURE 6. PLOT OF SHIFT FROM BASELINE IN PERCENT POWER VERSUS FREQUENCY BAND FOR CHANNEL 2 AT 90 MINUTES

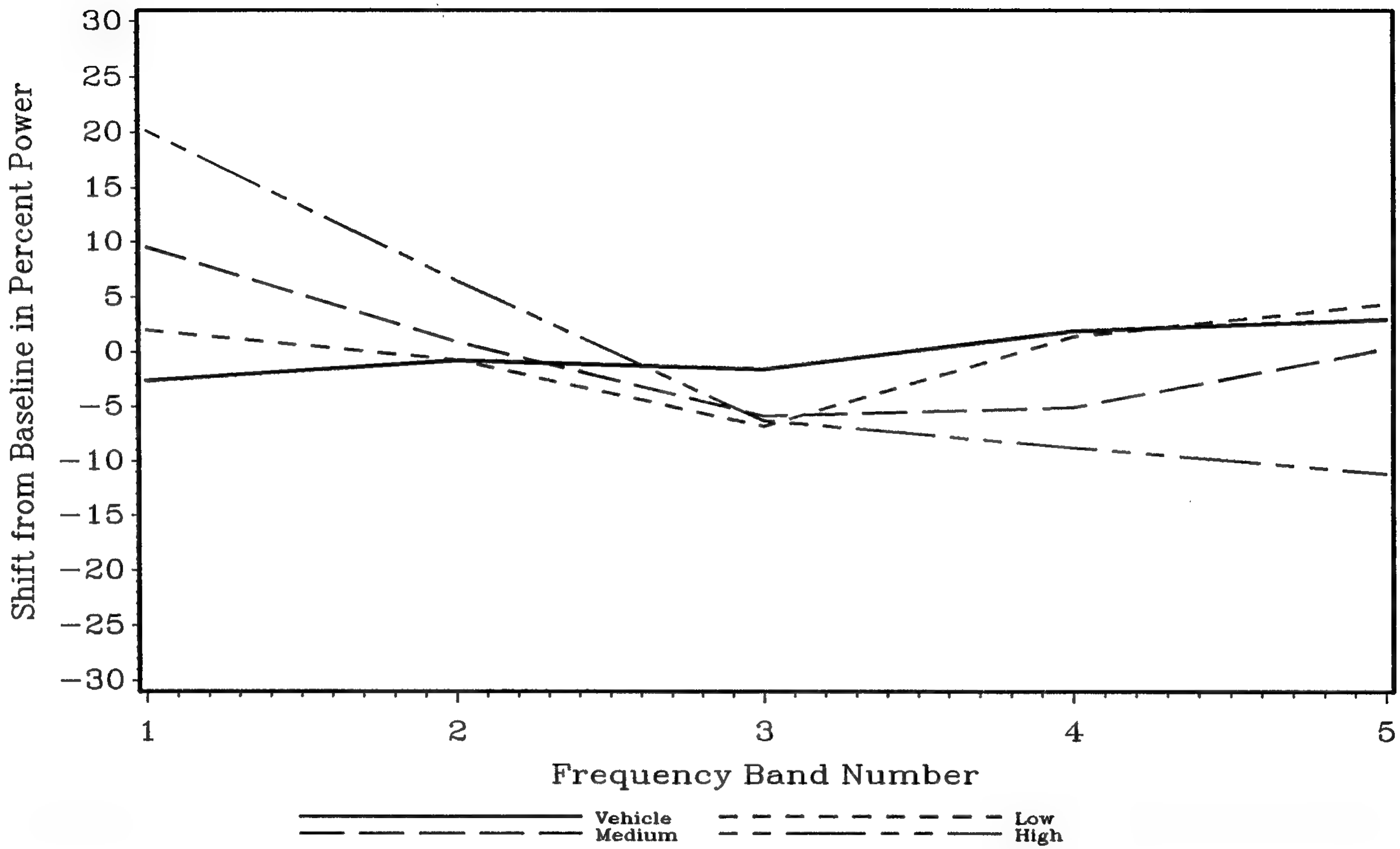

FIGURE 7. PLOT OF SHIFT FROM BASELINE IN PERCENT POWER VERSUS FREQUENCY BAND FOR CHANNEL 2 AT 300 MINUTES

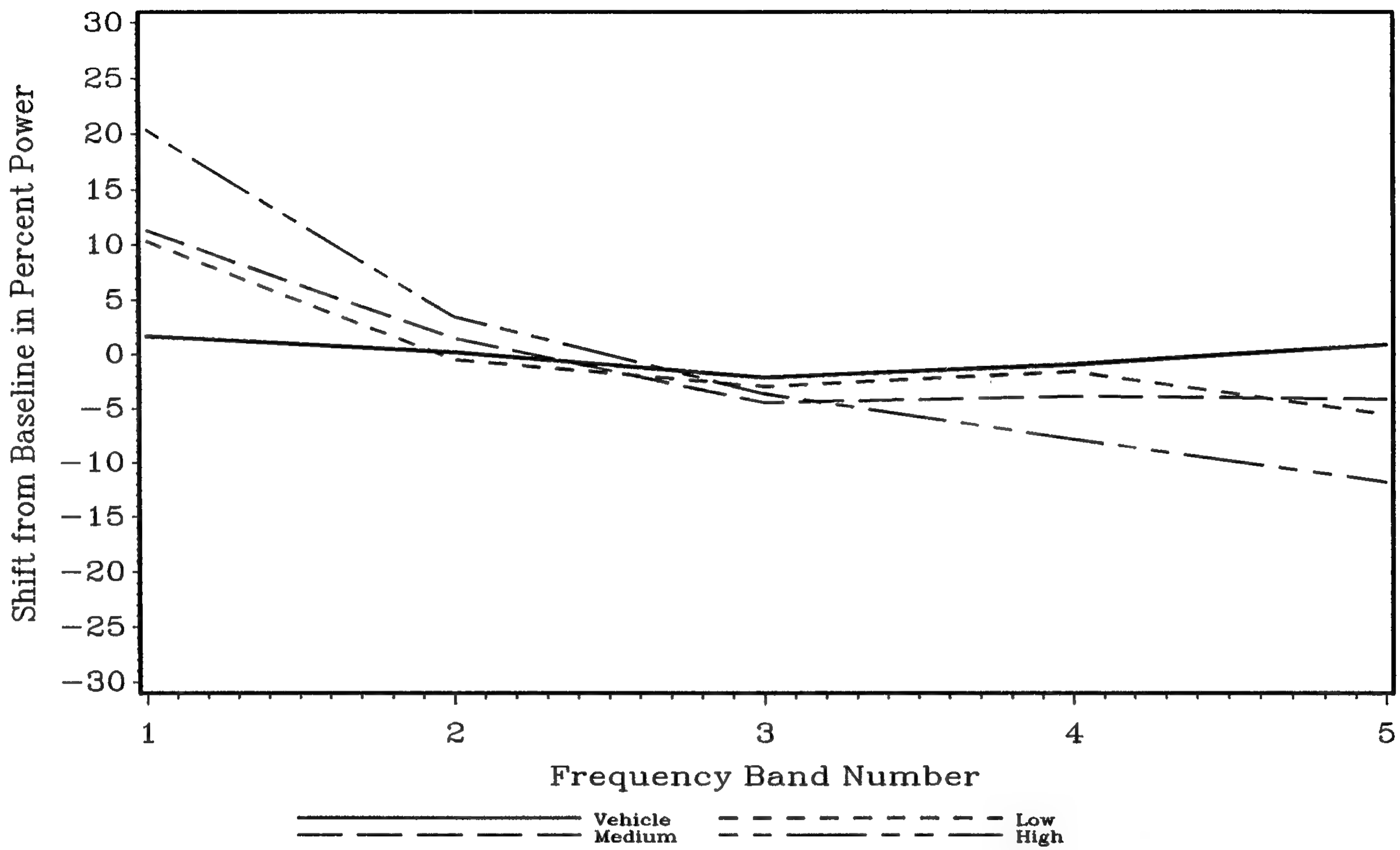

FIGURE 9. PLOT OF SHIFT FROM BASELINE IN PERCENT POWER VERSUS FREQUENCY BAND FOR CHANNEL 3 AT 30 MINUTES

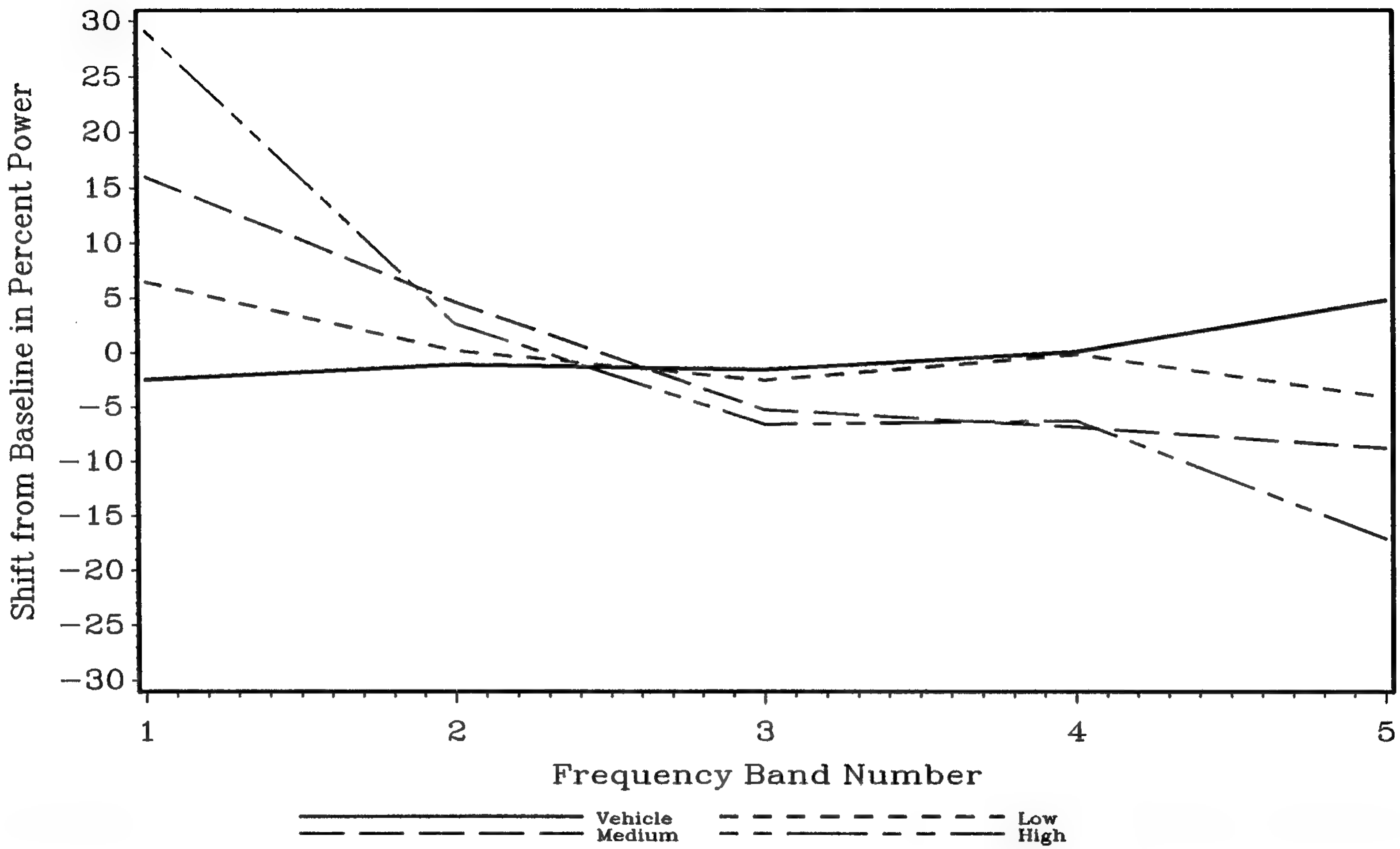

FIGURE 10. PLOT OF SHIFT FROM BASELINE IN PERCENT POWER VERSUS FREQUENCY BAND FOR CHANNEL 3 AT 90 MINUTES

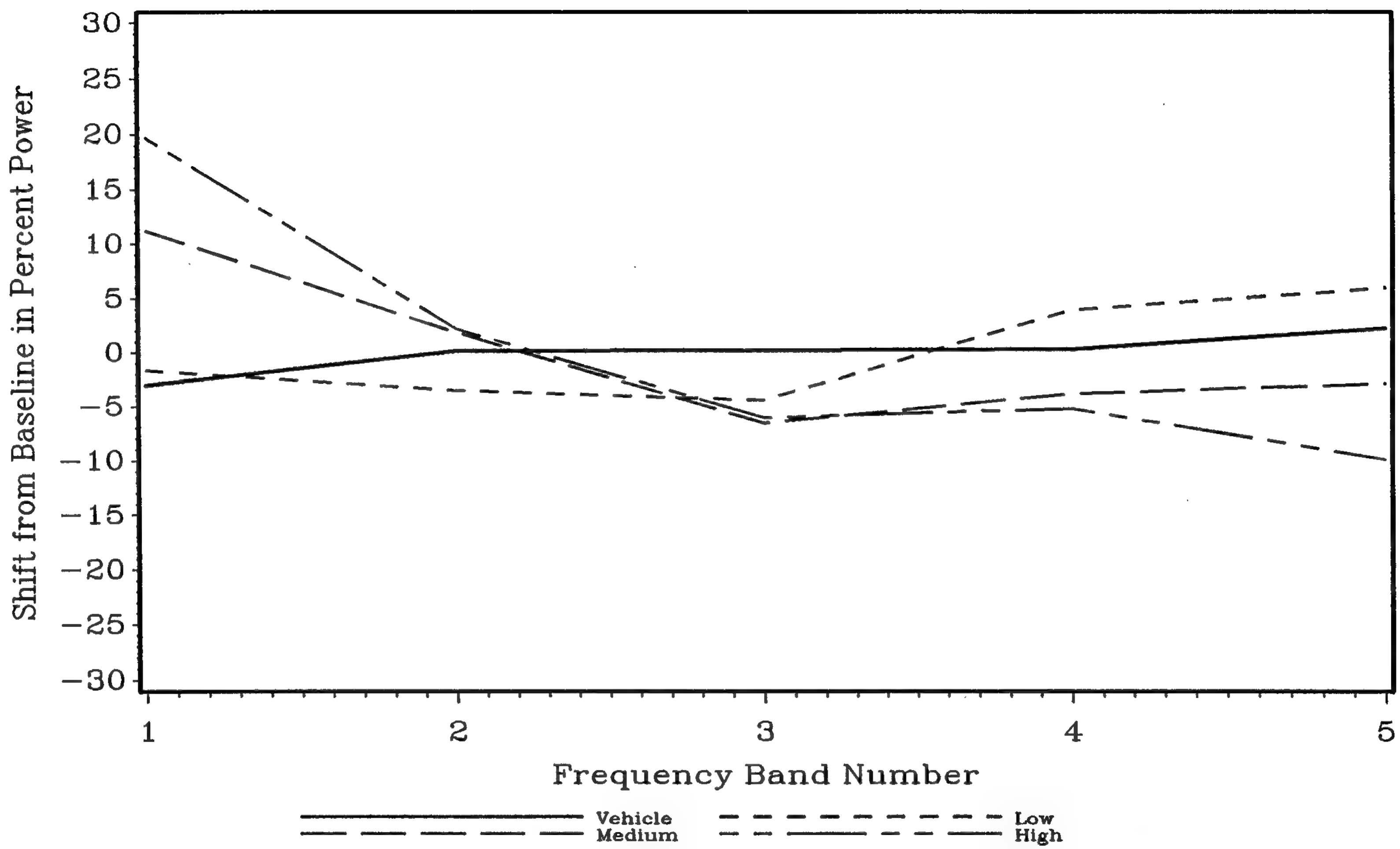

FIGURE 11. PLOT OF SHIFT FROM BASELINE IN PERCENT POWER VERSUS FREQUENCY BAND FOR CHANNEL 3 AT 300 MINUTES

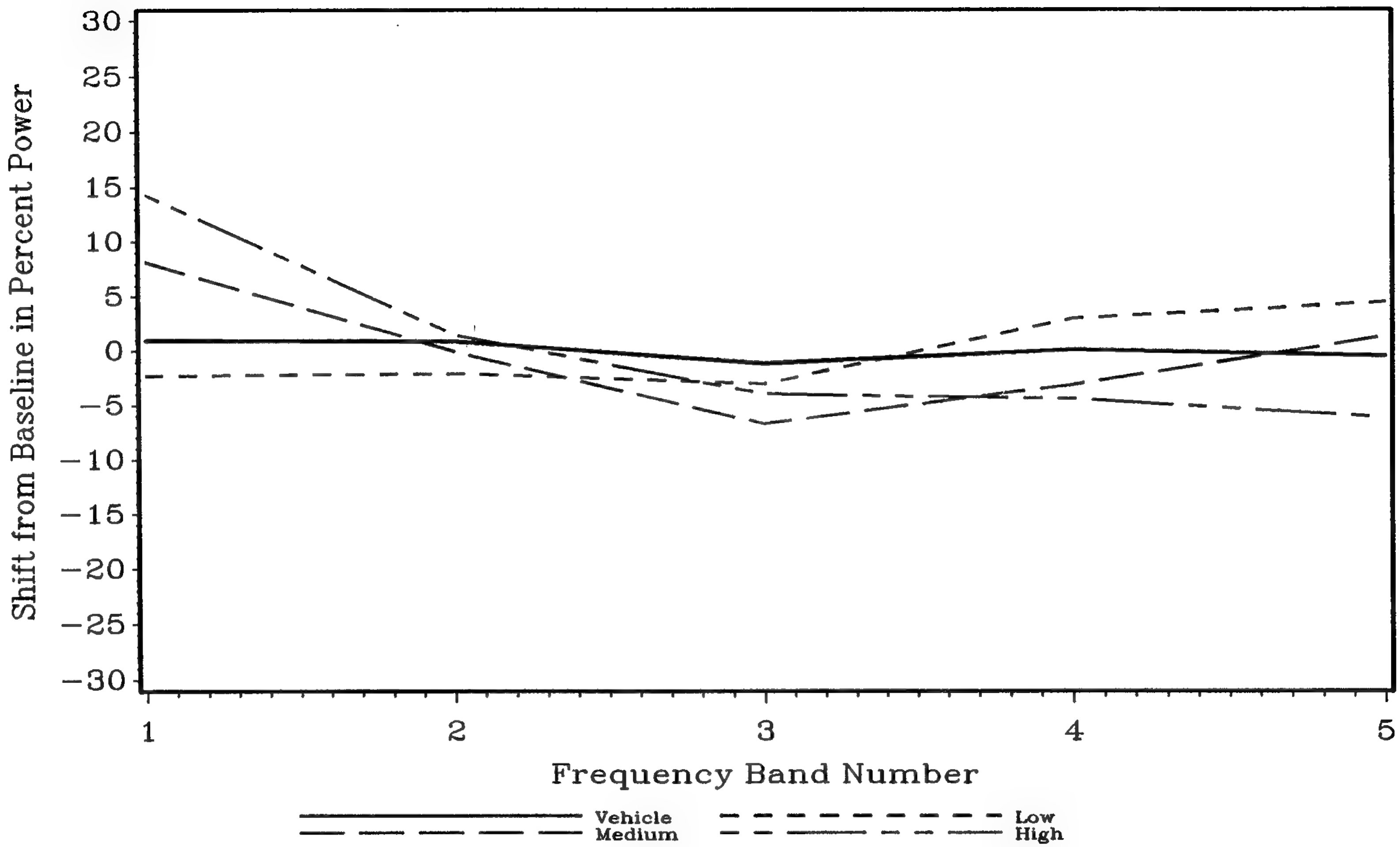

FIGURE 12. PLOT OF SHIFT FROM BASELINE IN PERCENT POWER VERSUS FREQUENCY BAND FOR CHANNEL 3 AT 600 MINUTES

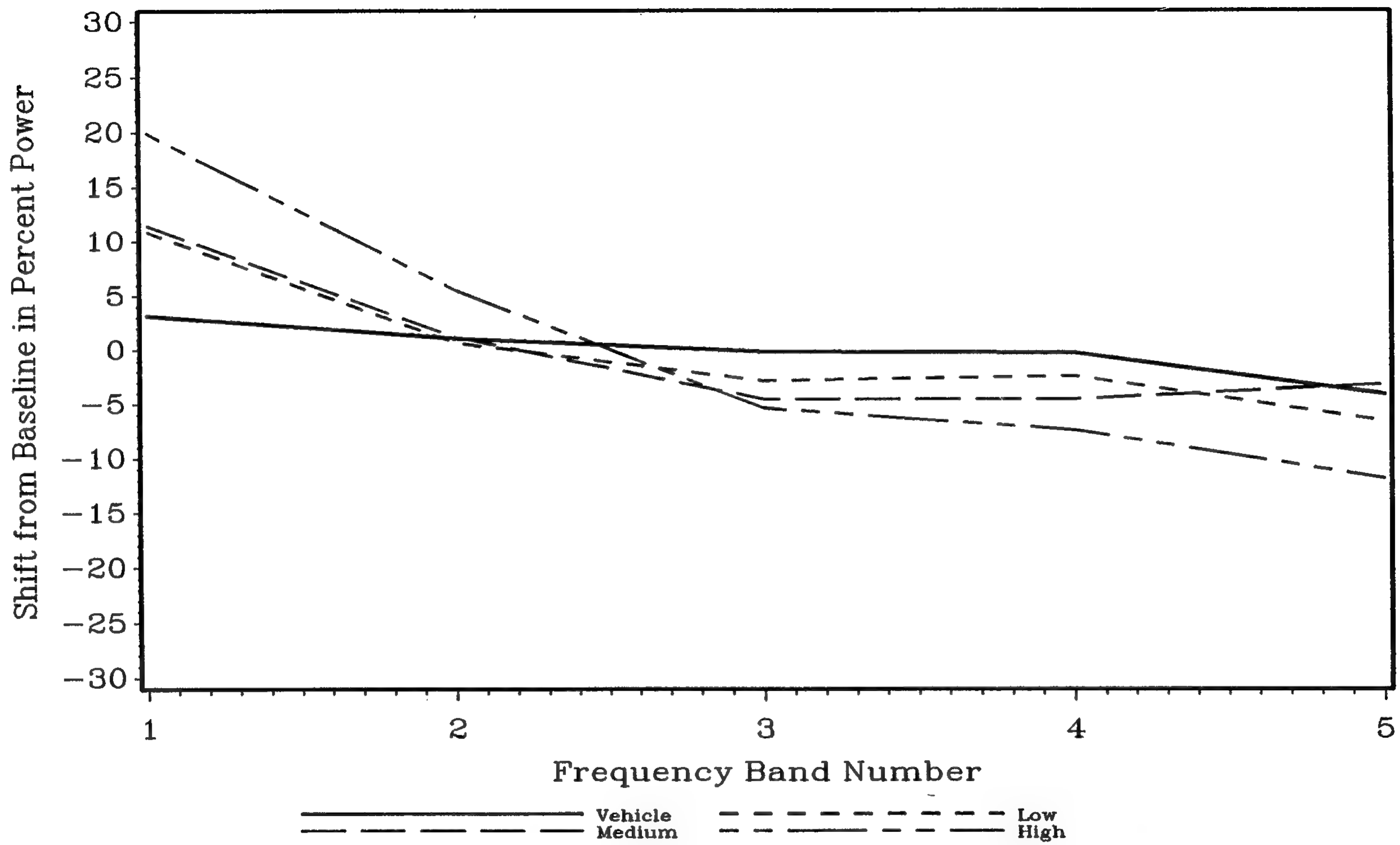

FIGURE 13. PLOT OF SHIFT FROM BASELINE IN PERCENT POWER VERSUS FREQUENCY BAND FOR CHANNEL 4 AT 30 MINUTES

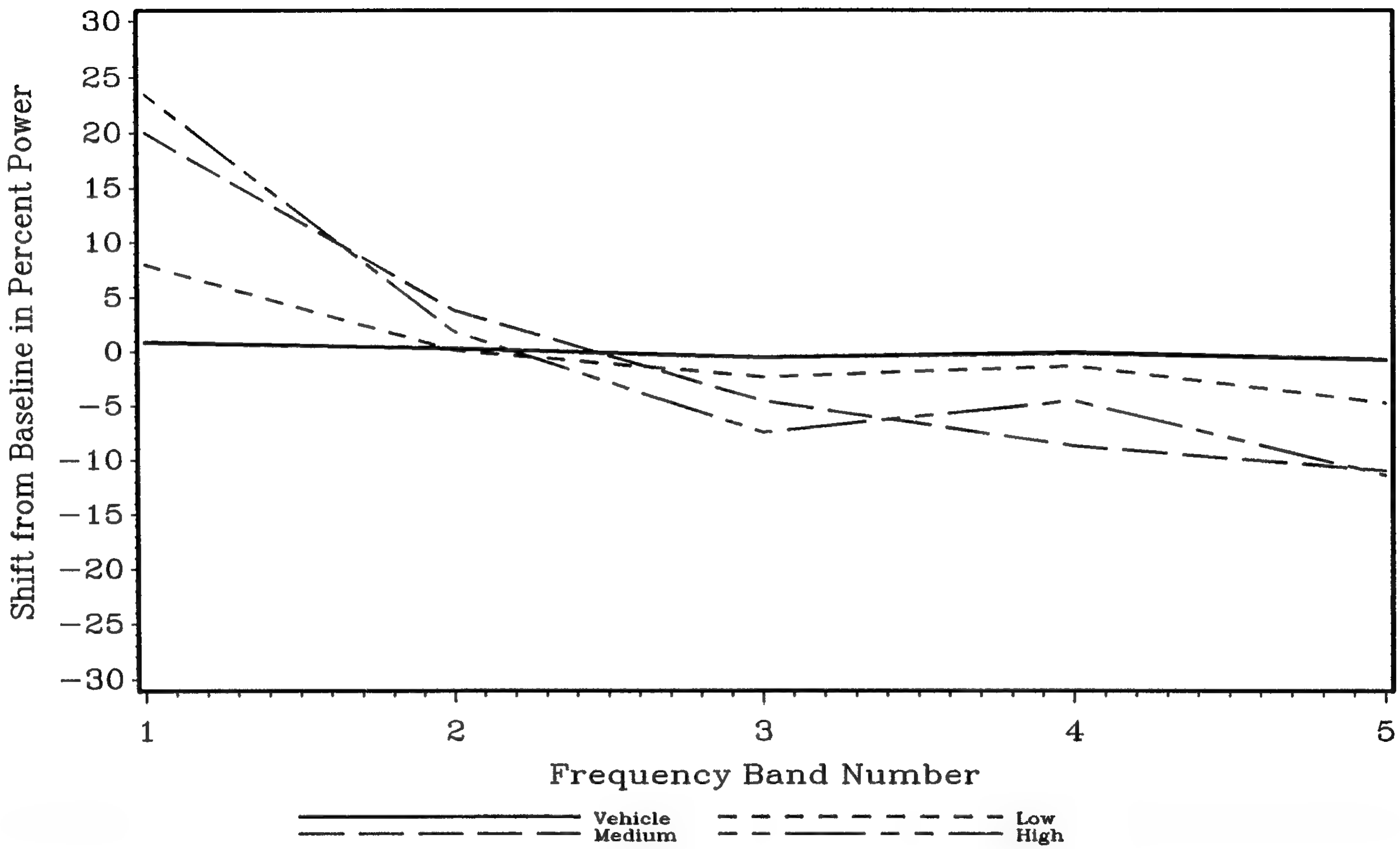

FIGURE 14. PLOT OF SHIFT FROM BASELINE IN PERCENT POWER VERSUS FREQUENCY BAND FOR CHANNEL 4 AT 90 MINUTES

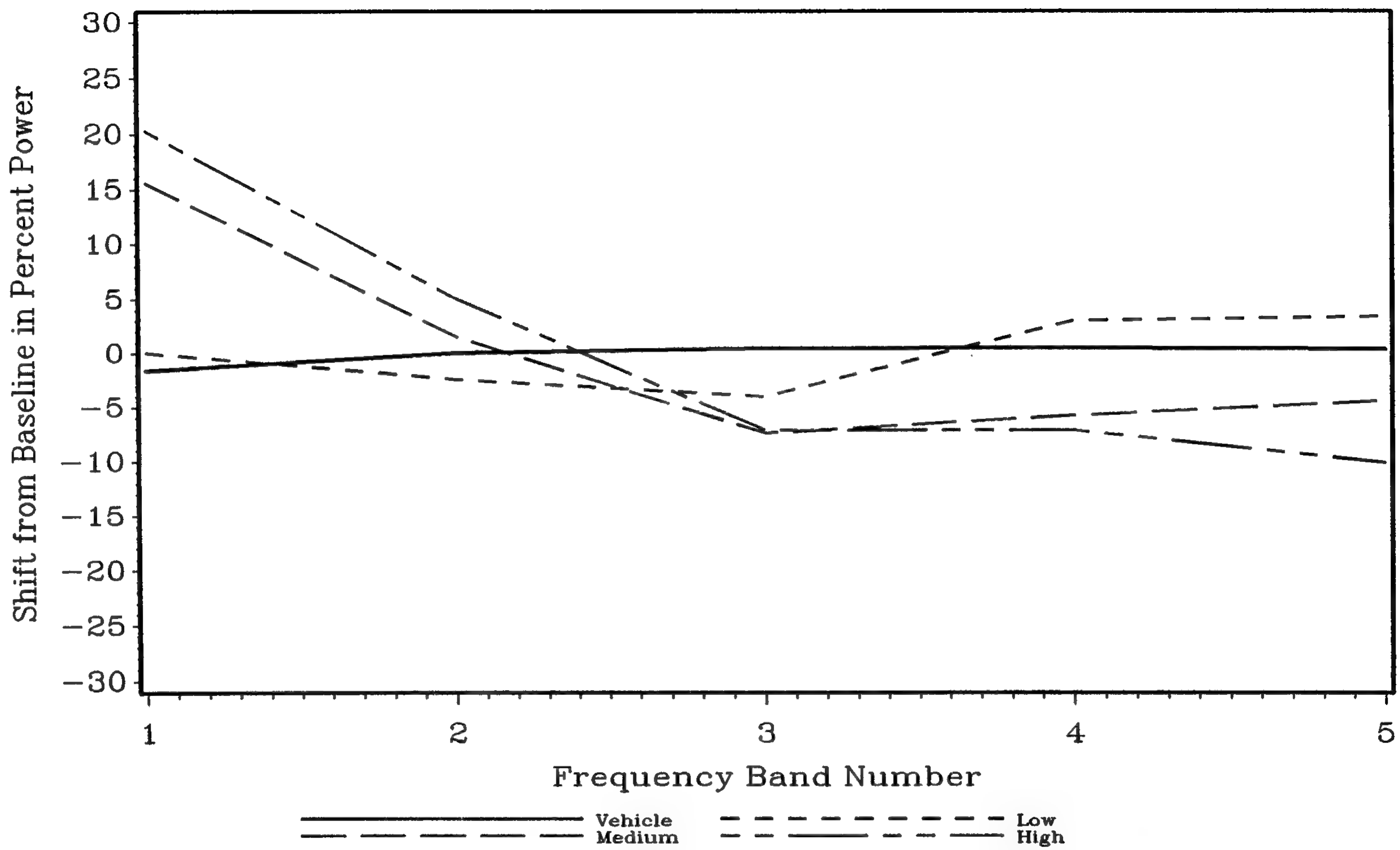

FIGURE 15. PLOT OF SHIFT FROM BASELINE IN PERCENT POWER VERSUS FREQUENCY BAND FOR CHANNEL 4 AT 300 MINUTES

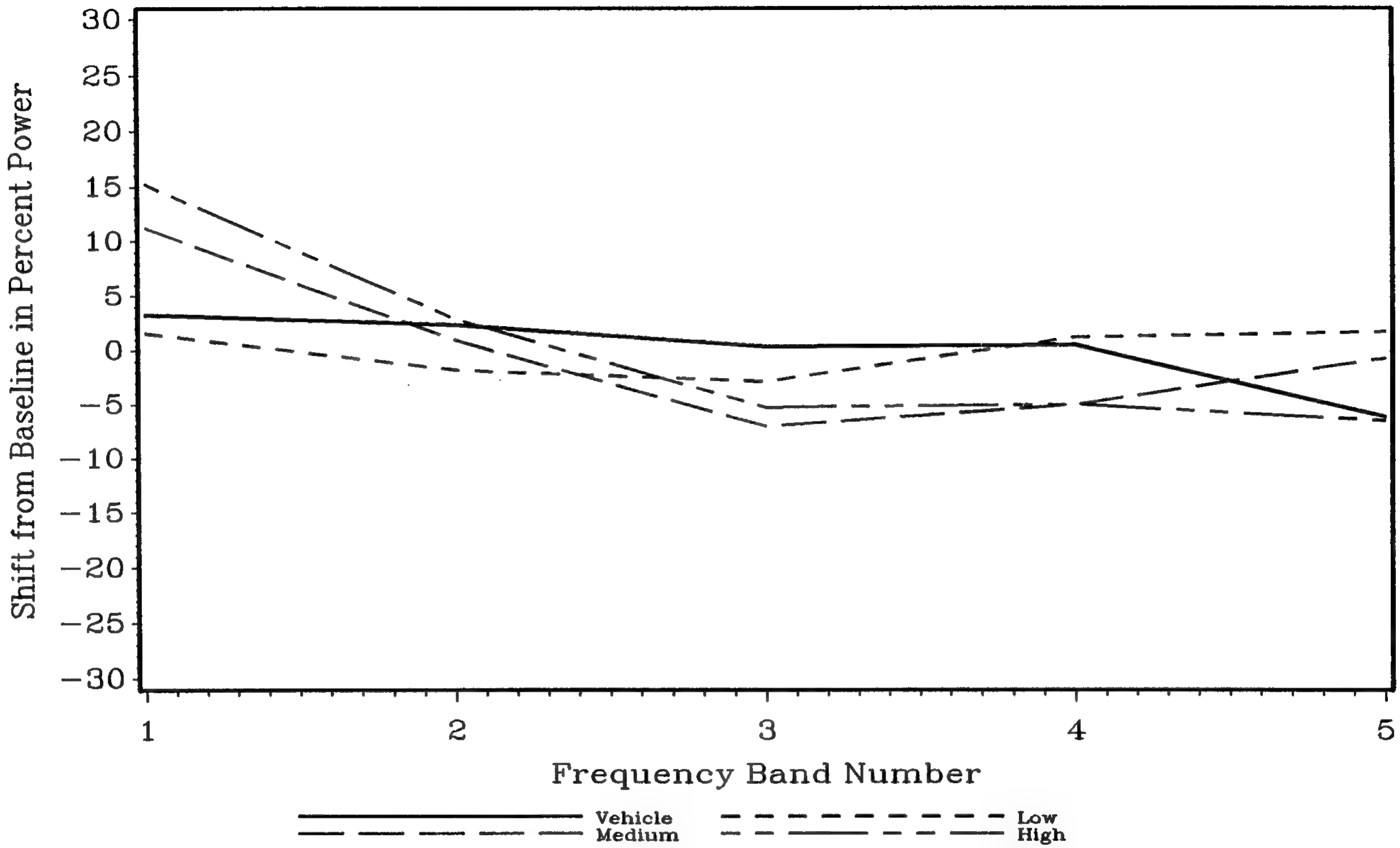

FIGURE 16. PLOT OF SHIFT FROM BASELINE IN PERCENT POWER VERSUS FREQUENCY BAND FOR CHANNEL 4 AT 600 MINUTES

(p-value < 0.05). In the 0.1 mg/kg atropine dose experiments, baseline shifts were mostly positive at 30 and 90 minutes. The results from 0.1 mg/kg dose experiments at 300 and 600 minutes did not demonstrate any general trend.

- Percent power readings in the first frequency band taken at the 30-minute time point after atropine administration are generally larger in magnitude than those taken at other time points.

- The difference between the atropine and saline control shifts from baseline is generally greater and tends to be more significant at the earlier time periods as compared to later time periods. Statistically significant differences in baseline shift between atropine and saline control occur less frequently at 300 and 600 minutes following dosing.

- Generally, statistical results for the four EEG channels are similar.

This expanded study of the dose-response relationship between atropine and EEG waveforms in rhesus monkeys confirms the increased percent power in the lower frequency bands that was demonstrated in the preliminary study of the high atropine dose (0.4 mg/kg). In addition, the results of this study demonstrate a change in the EEG waveforms of rhesus monkeys at a threshold atropine dose of 0.1 mg/kg. Although a trend was established which showed that the magnitude of the dose-response relationship diminishes with respect to time, there were not enough available data to establish either the duration of the effect of atropine on EEG waveforms or the time at which peak percent power in lower frequency bands occurs after atropine dosing.

4.3 CONCLUSIONS BASED ON THE EEG CONSULTANT'S REVIEW

The experimental results were reviewed by Dr. John R. Hughes, Director of the Department of Neurophysiology at the University of Illinois Chicago Medical Center. His review of the experimental results and summary of the relevant literature are presented in Attachment I. The following conclusions are based on Dr. Hughes' interpretation of the experimental results.

An increase in slow wave activity at 1-4 Hz is observed at an atropine dose of 0.1 mg/kg, increasing in magnitude at a dose of 0.2 mg/kg, and further increasing at a dose of 0.4 mg/kg. The higher frequency wave activity, especially 8-13 Hz, is decreased.

EEG slowing in monkeys was previously observed at an atropine dose of 1.5 mg/kg in non-computerized EEG studies. In the present, more elegant, computerized EEG study, slowing was observed at an atropine dose of 0.1 mg/kg. This 15-fold increase in sensitivity was attributed to the use of the computerized EEG analysis. EEG slowing in man has previously been observed at an atropine dose of 0.04 mg/kg in non-computerized EEG studies. An extrapolation based on the 15-fold increase in sensitivity of computerized EEG analysis leads to the conclusion that slowing in man may occur at an atropine dose of 0.003 mg/kg.

Divided attention in man has been reported previously at an atropine dose of 0.007 mg/kg, which is comparable to the extrapolated threshold atropine dose of 0.003 mg/kg. The increased slowing in brain activity expected to occur at this dose range of atropine is not expected to seriously impair the soldier. An atropine dose of 0.012 mg/kg, with its expected EEG slowing and increase in coordination errors, could significantly impair a soldier's performance of complex tasks. A dose of 0.028 mg/kg clearly produces EEG slowing in man (from direct evidence) and can affect helicopter flight performance in some individuals. An atropine dose of 0.040 mg/kg would likely place the soldier on the battlefield in jeopardy.

ATTACHMENT I

REVIEW OF EXPERIMENTAL RESULTS AND SUMMARY OF RELEVANT LITERATURE

by

Dr. John Hughes
Department of Neurophysiology, Director
The University of Illinois at Chicago Medical Center

EXTRAPOLATION OF PRESENT RESULTS TO MAN

A REVIEW OF THE MOST RELEVANT WORLD LITERATURE

Man

Dose. (mg)	(assume 70 kg person) (mg/kg)
0.50	0.007 impairs divided attention (Linnoila 1973)
0.85	0.012 increases coordination errors (Seppala & Visakorpi 1983)
1.05	0.015 no EEG change (Cozanitis 1983)
1.50	0.021 increases (computerized) EEG slowing (Pickworth et al. 1990)
2.00	0.028 at times affects flight performances (Caldwell et al. 1989)
2.80	0.040 increases EEG slowing, poor coordination (Itil & Fink 1968)
4.00	0.057 serious problems in flight performance (Caldwell et al. 1989)

Monkey (Baboon also)

--	1.50 alternating sleep-wake, EEG slowing (White et al. 1961)
--	2.00 agitation and EEG slowing (Meldrum et al. 1970)
--	2.00 " " " (Domino & Hudson 1959)

The present results in the monkey with a more elegant quantitative EEG than in past studies have lowered by 15 times the effective atropine dose for EEG slowing from 1.5 mg/kg in the non-computerized EEG to 0.1 mg/kg in the computerized EEG. This latter level is similar to the dose of 0.1 - 0.2 mg/kg in the cat (Lindsley, et al. 1968; Martin & Eades 1960; Torii & Wikler 1966; Vilablanca 1967) and 0.1 mg/kg in the dog (Wikler 1952) for non-computerized EEG slowing.

The apparent threshold for non-computerized EEG slowing in the monkey is 1.5 mg/kg and in man is 0.04 mg/kg. If the 15X difference between the lowest dose of EEG slowing in non-computerized vs. computerized records in the monkey could be extrapolated to man, the 0.04 mg/kg for non-computerized slowing converts to 0.003 mg/kg that could also produce slowing in computerized studies. This latter dose is less than the 0.021 mg/kg that Pickworth et al. found produced (computerized) slowing, but lower doses were not investigated. This extrapolated dose of 0.003 mg/kg that would be expected to produce computerized EEG slowing in man is of an order of magnitude similar to the 0.007 mg/kg dose that is known to produce the psychological change of impaired divided attention. The

lower threshold for an EEG change than for a clinical change is often seen in neurophysiological clinical correlations, especially when elegant computerized analyses are used to detect subtleties in the EEG.

The peak of the EEG slowing with the low dose of 0.1 mg/kg was generally at 30 min and lasted for 600 min, although in some animals the duration was only 90 min. The peak at the medium dose of 0.2 mg/kg was usually at 90 min and for the high dose of 0.4 mg/kg was more variable between 30, 90, and 300 min, but the duration for both was usually 600 min. From Tables 2-11, the magnitude of EEG change at 30 min from the low to the medium dose was 100 percent to 123 percent (mean) and from the low to the high dose was to 213 percent (mean). Thus, the change from 0.1 to 0.2 mg/kg added only about 1/4 more slowing, but from 0.1 to 0.4 mg/kg, more than doubled the slowing at 30 min. At 90 min the magnitude of EEG change from 0.1 to 0.2 mg/mg was 100 percent to 203 percent and from 0.1 to 0.4 mg/kg was to 285 percent (mean). Thus, at 90 min, the change from 0.1 to 0.2 mg/kg was double, and from 0.1 to 0.4 mg/kg was almost triple when the indices seen in these tables are used for magnitude of changes.

Tables in the main report show similar results whether the data are differences from baseline (Table 2), differences between doses and controls (Table 3), in the 4 separate channels (Table 4-7), differences in baseline shifts between doses and controls in the 4 separate channels (Tables 8-11), differences in the first band in 6 different animals (Tables 12-17), or differences in baseline shift in 4 different animals (Tables 18-21).

For the low dose of 0.1 mg/kg, the maximal increase in both Bands 1 and 2 is noted at 30 min (9 tables) or 90 min (6 tables) with 3 exceptions when Band 2 is similar to Bands 3-5 showing a decrease in activity.

For the medium dose of 0.2 mg/kg, the minimal <u>increase</u> in both Bands 1 and 2 is noted at <u>90 min</u> (11 tables) or 30 min (3 tables), rarely at 300 min (3 tables) or 600 min (1 table). Bands 3-5 show a decrease in activity.

For the high dose of 0.4 mg/kg, the maximal increase in both Bands 1 and 2 is noted at 30 min (8 tables), 90 min (12 tables), 300 min (8 tables) or 600 min (3 tables). Bands 3-5 show a decrease in activity.

Thus, in all three doses, an increase is seen in Bands 1 and 2 and a decrease in Bands 3-5. The maximal increase tends to appear at 30 min for the low dose, 90 min for the medium dose and at 90-300 min for the high dose. These generalizations are confirmed in Figures 1-16. These latter figures demonstrate well that the decrease in activity from Bands 3-5 is maximal, usually in Band 5.

In summary, an increase in slow wave activity at 1-4 Hz is seen (to a lesser extent at 4-8 Hz) with a low atropine dose of 0.1 mg/kg, increasing in effect at a medium dose of 0.2 mg/kg and further increasing at the high dose of 0.4 mg/kg. The higher frequencies, especially at 8-13 Hz, show a decrease in activity (to a lesser extent the frequencies of 13-18 and 18-25 Hz). With increasing dose from 0.1 to 0.2 to 0.4 mg/kg, the peak of the increase in slowing tends to be later, from 30 min (0.1) to 90 min (0.2) to a variable time of 30-90-300-min (0.4) with these three increasing doses.

Final Conclusions

1. This further analysis of data under Contract No. DAMD17-89-C-9050 confirms the data from the earlier pilot study that showed an increase in slow waves with the atropine dose of 0.4 mg/kg in the monkey. The present data demonstrate that slow waves are also seen with the lower doses of 0.2 mg/kg and 0.1 mg/kg.
2. The dose of 0.1 mg/kg most often showed a peak of EEG slowing at 30 min, 0.2 mg/kg at 90 min and 0.4 mg/kg more variable at 30-90-300 min. The duration of significant change for 0.1 mg/kg was 90 min for some animals, 600 min for others and for 0.2 and 0.4 mg/kg was generally 600 min.
3. For the magnitude of change at 30 min, the dose of 0.2 mg/kg added about 1/4 more slowing to that from the dose 0.1 mg/kg and 0.4 mg/kg more than doubled the amount. At 90 min the change from 0.1 to 0.2 mg/kg doubled, and to 0.4 mg/kg nearly tripled the amount of slowing (using the indices in Tables 2-11).
4. From the literature, comparable non-computerized EEG and clinical changes have been seen in the monkey at 1.5 mg/kg and in man at 0.04 mg/kg (37.5 times difference). Since the threshold for slowing in the monkey is reduced from 1.5 to

0.1 mg/kg (15X difference) by the present computerized studies, a comparable change in man would be from 0.04 to 0.003 mg/kg, as the lowest threshold for EEG slowing.

5. If the assumptions are made (1) that the threshold for non-computerized slowing in the monkey was determined in the same way as the threshold in man and (2) that changes in that threshold for computerized slowing in the monkey can be extrapolated to man, the following conclusions may be drawn for man:
   (1) Slow waves may appear with a dose of atropine of 0.003 mg/kg, with a peak effect usually at 30 min, in some lasting 90 min, in others 600 min.
   (2) Slow waves would appear with a dose of 0.006 mg/kg with a peak effect usually at 90 min, usually lasting 600 min. At 30 min the amount of slowing would be only 1/4 more than the dose of 0.003 mg/kg, but at 90 min would likely be double.
   (3) Slow waves would appear with a dose of 0.012 mg/kg with a variable peak from 30-300 min, usually lasting 600 min. At 30 min, the amount of slowing would be nearly double and at 90 min nearly triple the amount from the 0.003 mg/kg dose.
6. The calculated threshold for slow waves in man of 0.003 mg/kg is of the same order of magnitude as the dose of 0.007 mg/kg that can impair divided attention.
7. The dose of 0.012 mg/kg can increase coordination errors.
8. The dose of 0.028 mg/kg can affect the accuracy of flight performance in helicopters in some individuals and is known from other independent data to produce EEG slowing in man.
9. The dose of 0.057 mg/kg can cause serious problems in the accuracy of flight performance and could be expected to significantly affect the performance of a soldier on the battlefield.
10. On the question of a "safe" dose of atropine for the soldier on the battlefield, we can only speculate. The calculated dose of 0.003 mg/kg for EEG and the effect of 0.007 mg/kg on divided attention would likely not seriously impair the soldier. The dose of 0.012 mg/kg with its expected increased EEG slowing and increased

coordination errors could significantly impair the soldier in complex tasks. Evidence shows that the dose of 0.028 mg/kg clearly produces EEG slowing and can affect (helicopter) flight performance in some individuals, who then would likely have limited effectiveness on the battlefield. The dose of 0.040 mg/kg would likely place the soldier on the battlefield in clear jeopardy and would result in ineffective performance.

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